EFFECT OF MICROCYTIC ANEMIA ON GLYCOSYLATED HEMOGLOBIN A_1c IN NON- DIABETIC ADULTS

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CERTIFICATE

This is to certify that the dissertation titled **"EFFECT OF MICROCYTIC ANEMIA ON GLYCOSYLATED HEMOGLOBIN A_1c IN NON- DIABETIC ADULTS"** is a bonafide work done by **DR.B. GOKHULA RAJ**, Post graduate student, Institute of Internal Medicine, Madras Medical College, Chennai -03, in partial fulfillment of the University Rules and Regulations for the award of Degree of MD General Medicine (Branch – I), Internal Medicine, under our guidance and supervision, during the academic year 2014 – 2017.

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DECLARATION

I solemnly declare that the dissertation titled "**EFFECT OF MICROCYTIC ANEMIA ON GLYCOSYLATED HEMOGLOBIN A_1c IN NON- DIABETIC ADULTS"** is done by me at Madras Medical College, Chennai – 600 003 during the period April 2016 to September 2016 under the guidance and supervision of **Prof**.**Dr.G. SUNDARAMURTHY** submitted to the Tamilnadu Dr. M.G.R Medical University towards the partial fulfillment of requirements for the award of M.D. DEGREE IN GENERAL MEDICINE (BRANCH-I) .

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ABBREVIATIONS

ADA	=	American Diabetes Association
DM	=	Diabetes Mellitus
HbA_1c	=	Hemoglobin A_1c (glycosylated hemoglobin)
FPG	=	Fasting Plasma Glucose
PPBG	=	Post Prandial Blood Glucose
OGTT	=	Oral Glucose Tolerance Test
RBC	=	Red Blood Cell
ATP	=	Adenosine Tri Phosphate
NADP	=	Nicotinamide Adenine Dinucleotide Phosphate
MAHA	=	Micro Angiopathic Hemolytic Anemia
WHO	=	World Health Organization

CONTENTS

INTRODUCTION

INTRODUCTION

Diabetes mellitus refers to a metabolic disorder with various etiologies which is characterized by chronic hyperglycaemia causingdisturbances of carbohydrate, fat and protein metabolism, resulting from relative or absolute deficiency of insulin or both. The long–termdeleterious effects of diabetes include development of microvascular complications like retinopathy (18%), nephropathy (23.2%), neuropathy (26%) and macrovascular complications like cardiac (21%), peripheral arterial (6.3%) and cerebrovascular disease (24.4%).

Since diabetes is now affecting the major workforce of various populations all over the globe, it has major anddeleterious impact on the productivity of an individual as well as the nation. Diabetes and its complications has atremendousnegative impact on the economic potential of both the developed and the developing nations.

This rises the issue of early diagnosis of diabetes in order to prevent the development of complications in order to improve the quality of life of the population.

The potential utility of HbA_1c in the diagnosis of diabeteswas first mentioned in the WHO report published in 1985.With more information regarding the diagnosis of diabetesbecame available, WHO along with IDF called upon for a joint expert meeting to review and update the

recommendations on diagnosis of diabetes mellitus in 2005 and to include HbA_1c in the diagnostic armamentarium.

Glycosylated haemoglobin (HbA_1c) is produced by a ketoamine reaction occurring between glucose molecule and the valine in the N-terminalend of ß-chains of the haemoglobin molecule. HbA1c levels helpclinicians to get an overall picture of the average blood sugar levels over a period ofweeks/months[1].

This is important for diabetics, because,the higher the HbA_1c levels, greater the risk of developing complications of diabetes.Chronic hyperglycaemic states tends to elevate the values of $HbA_1c$and it correlatespositively with the metabolic control[2]. According to theguidelinespublished by the American Diabetes Association (ADA), HbA_1c should be kept < 7% in patients with diabetes. The values > 7% indicate an increased probability of diabetes – related complications, especially microvascular.

When there is chronic hyperglycemia, the nonenzymaticglycation of haemoglobin increases. This nonenzymatic glycation of proteins occurring as a result of hyperglycemia,has pronounced effects on the structure and functions of proteins. The two factors which can affect the glycation of proteins are theconcentration of blood glucoseandthe half life of the protein[3], the latter being constant as it is genetically determined.

Thus, the quantity of HbA_1c is determined solely by the blood glucose concentration.

Many studies have shown that the level of HbA_1ccan be altered by various other factors apart from blood glucose values. One such factor is the quantity of iron in the blood. So, iron deficiency anemia, which is the most common of the microcytic anemias[4] plays a significant role in the measurement of HbA_1c. Thus, India being the diabetic capital of the world, along with microcytic anemia prevailing so common in the community, needs certain measures to prevent false reporting of diabetic cases.

Thus, the main aim of our study was to determine whether the level of HbA_1c increased/decreased among the anemic patients without diabetes. If so, theanemia has to be corrected before any diagnosticor therapeutic decision was made based on the HbA_1c levels.

AIMS AND OBJECTIVES

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1. To estimate HbA_1c levels in non-diabetic adults with microcytic anemia.

2. To compare it with HbA_1c levels of non-diabetic controls and thereby establish a correlation between MCV and HbA_1c levels.

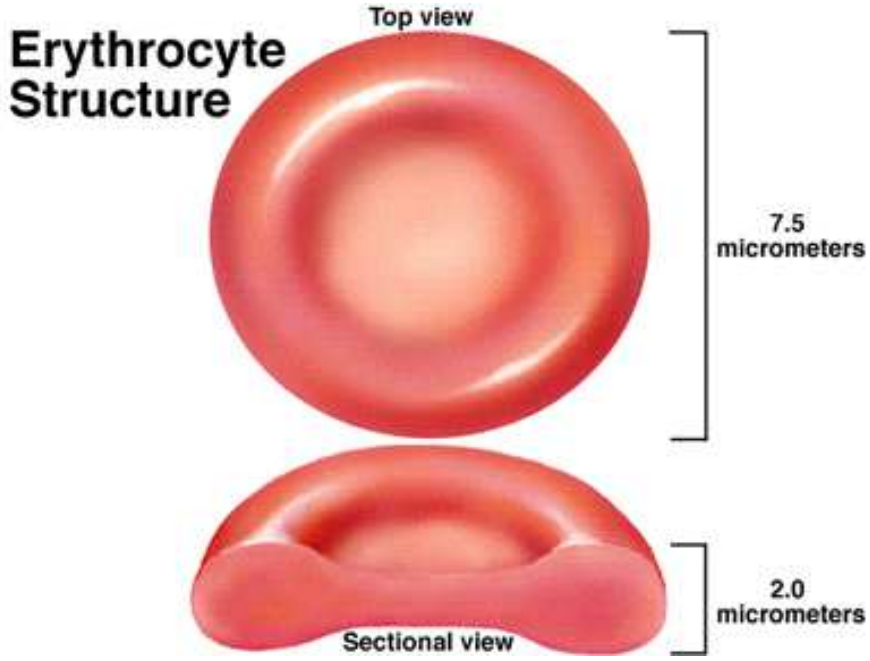
REVIEW OF LITERATURE

REVIEW OF LITERATURE

THE RED BLOOD CELL

Mature erythrocytes are unique of all the cells of human tissues, because they lack nucluei and cytoplasmic structures like lysosomes, endoplasmic reticulum, mitochondria, golgi apparatus[8] etc., Hence they are incapable of undergoing mitosis, protein synthesis or oxidative reactions.

They have a diameter of 7-8 microns and normally exist in the bi-concave form. Their membranes are so elastic that they can squeeze through the capillaries attaining lesser diameters than the bi-concave structure and can rapidly resume their original structure.



MEMBRANE AND CYTOSKELETON:

The membrane of the erythrocyte is highly deformable. It is fragile, but its integrity is maintained by the cytoskeletal structures on its inner surface, which forms a lattice like structure scaffolding the red cell membrane, thus maintaining the shape of the red cell. The *fluid mosaic model* best explains the structure of the red cell memrane, consisting of a lipid bilayer which is traversed by several trans-membrane proteins.

Lipids:

Most of the membrane lipids of the erythrocyte are either phospholipids or unesterified cholesterol. The phospholipids include aminophosphatides (phosphatidylethanolamine and phosphatidylserine) on the inner side and choline containing lipids (phosphatidylcholine and sphingomyelin) on the outer side. Cholesterol plays an important role of maintaining the fluidity of the membrane, thus making the membrane more viscous.

Proteins:

Transmembrane proteins:

The two important proteins in the erythrocyte cell membrane include:

- Anion exchanger protein 1 (AE1), also called as band 3 protein
- Glycophorin A (GPA)

AE1 protein takes care of the facilitated transport of anions and glucose into the RBC. The extra-cellular domain of AE1 protein is heavily glycosylated and it bears the carbohydrate blood group antigens (ABO and I/i blood group antigens)[9]. AE1 protein also interacts with ankyrin and contributes to membrane stability.

The GPA protrudes to the exterior surface, contributing much to the negative charge of the outer surface of the red cell membrane at physiological pH[10]. It bears the M and N blood group antigens and is the binding site for antigens of Plasmodium falciparum

Other trans-membrane proteins include:

- Rh proteins
- glycophorin B which carries S/s blood group antigens.

Cytoskeletal proteins:

The important cytoskeletal proteins in the erythrocyte membrane include:

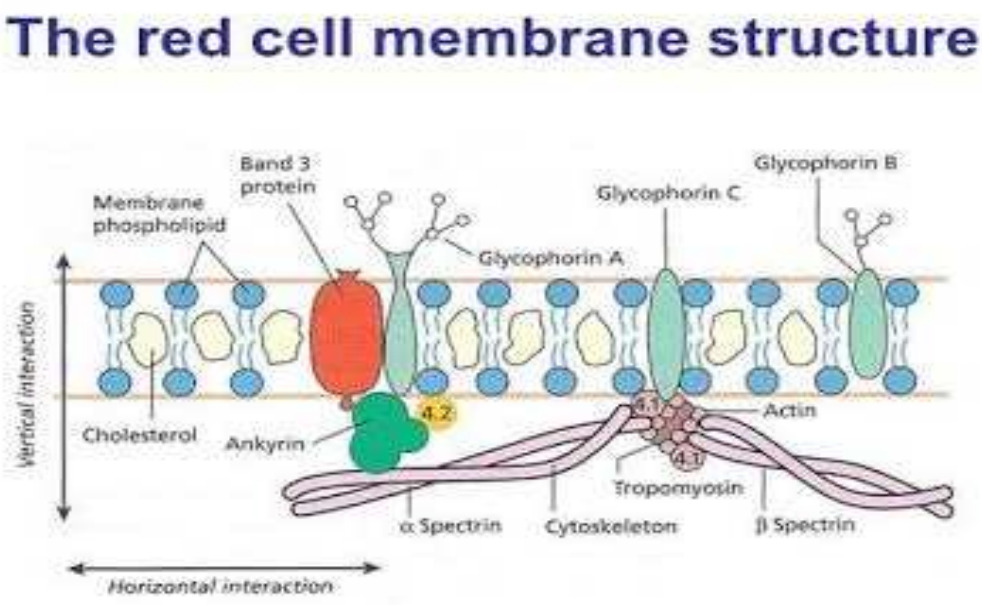
- Spectrin
- Ankyrin
- Protein 4.1

Spectrin is intertwined among itself and actin to form a lattice like network attaching to the inner surface of the red cell membrane. This

structure is a resilient structure that helps the RBC to resume its biconcave disc shaped structure after forces causing distortion of the membrane are removed[11].

Other minor cytoskeletal proteins include:

- Protein 4.9
- Tropomyosin
- Tropomodulin
- Adducin



CYTOPLASM:

The major junk of the RBC cytoplasm is made up of haemoglobin. It contributes to about 90% of the dry weight of the RBC[12]. The next important cytoplasmic protein is the enzyme, carbonic anhydrase.

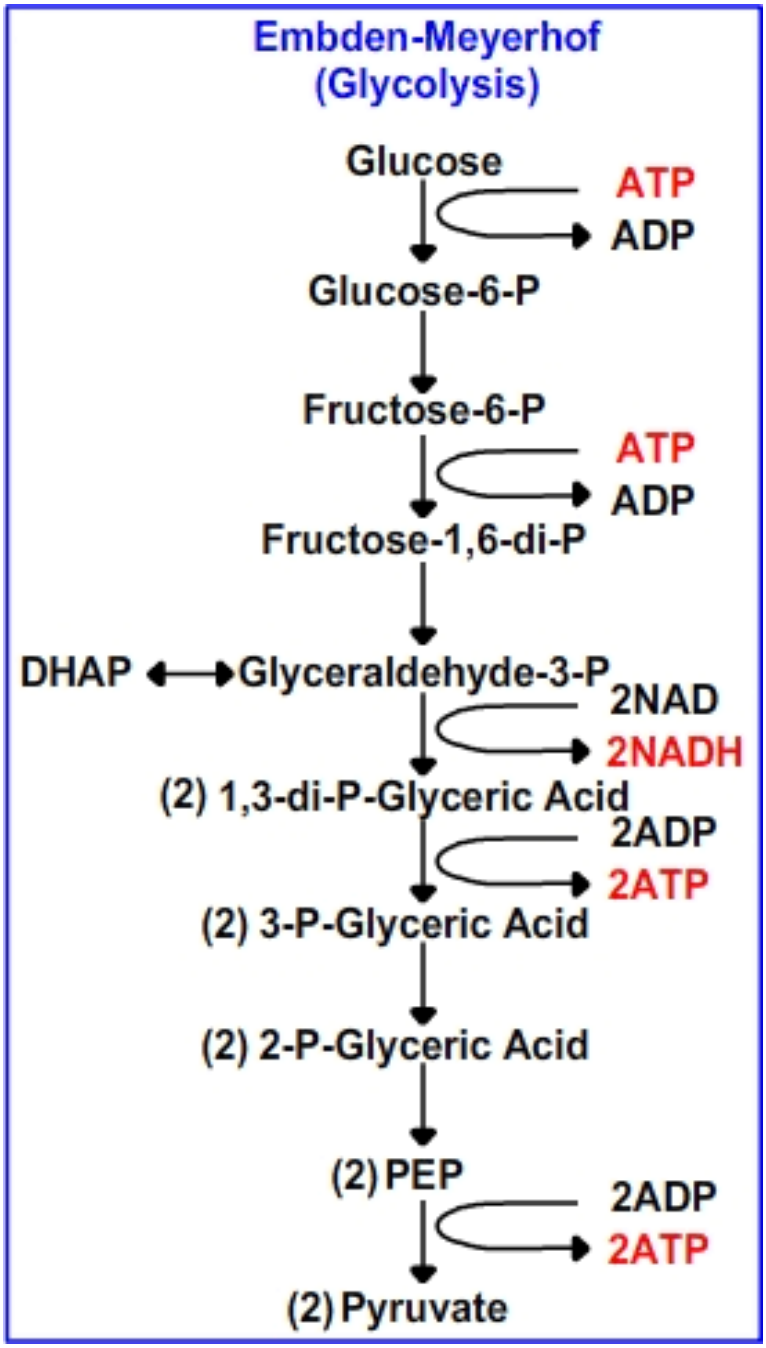
The structure, functions and the bio-chemistry of haemoglobin molecule is discussed in detail later.

Metabolic processes inside the erythrocyte:

The survival of the RBC depends upon their capacity to generate high energy Adenosine TriPhosphate (ATP) and reduced dinucleotides.

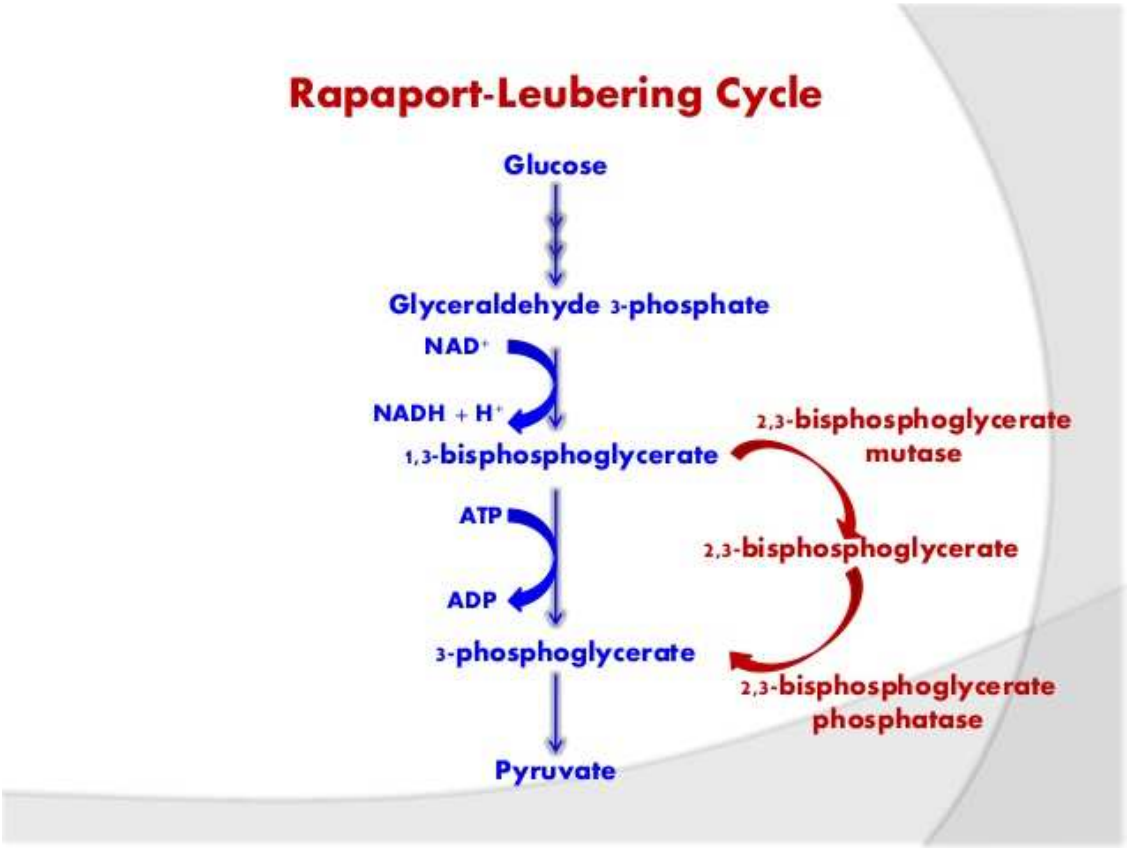
Since the erythrocytes lack mitochondria, it is not possible for Kreb's cycle to take place, so aerobic glycolysis does not occur. Instead, ATP is generated through the anaerobic glycolytic (Embden-Meyerhoff) pathway[13].

A constant supply of ATP is required for the RBC to keep the Na^+-K^+ ATPase pump active, in order to drive out the Na^+cation out of the cell and maintain the influx of K^+ ion. Thus, Na^+ ion iside the cell is extruded preventing hemolysis.



Thus the EMP pathway results in the production of only 4 molecules of ATP and 2 molecules of NADP, which is significantly less than that produced from the aerobic glycolytic pathway[14].

A large amount of 2,3 BPG is present in the erythrocytes, which is produced as a result of anaerobic glycolytic pathway. The rate of synthesis and degradation of 2,3 BPG is regulated by the Rapaport-Luedberg shunt.



2,3 BPG plays an important role in regulating the oxygen binding capacity of the haemoglobin molecule.

A small amount of glucose entering the RBC is utilised in the pentose phosphate pathway. NADP (Nicotinamide Adenine Dinucleotide Phosphate) is the major product of this pathway.

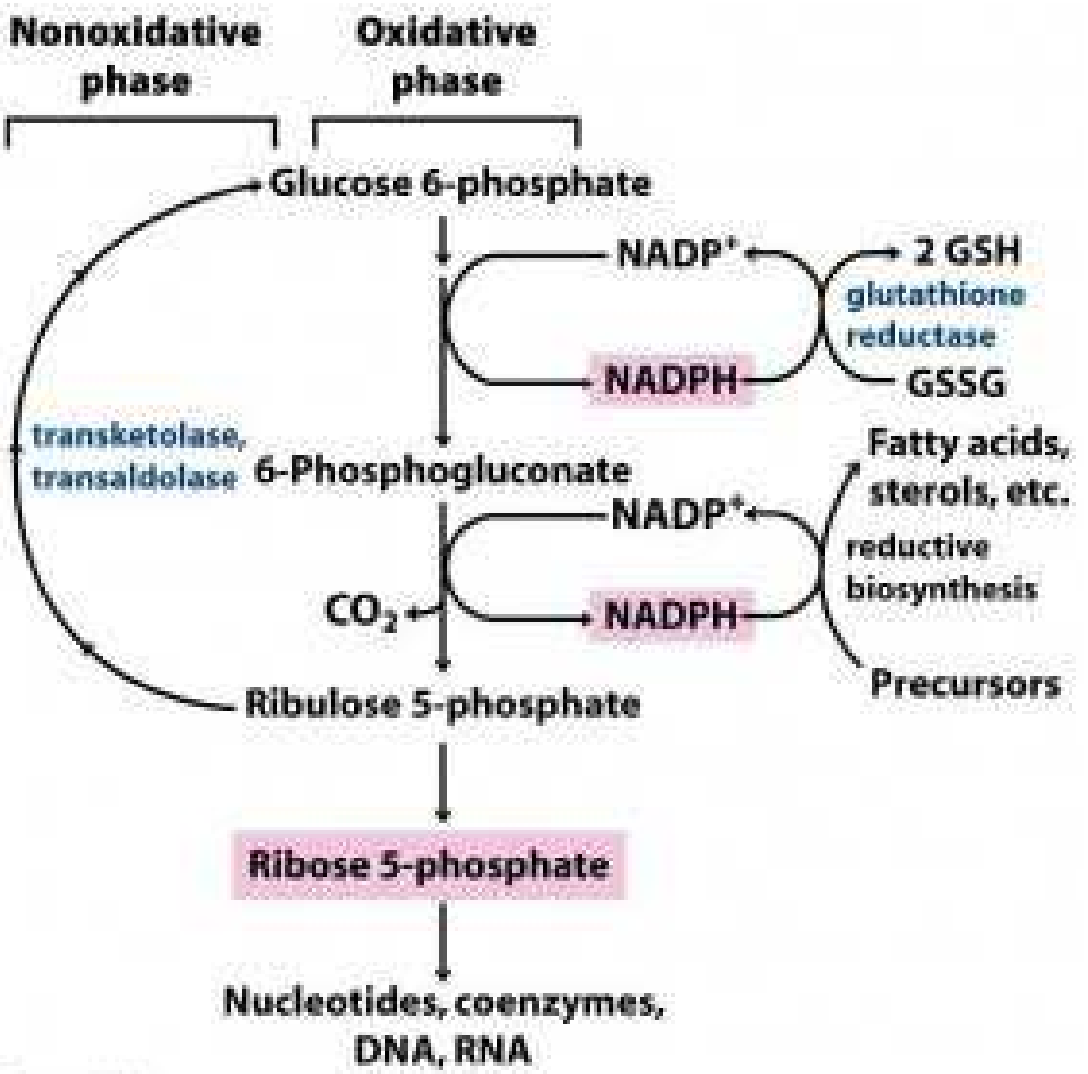


Figure 14-20
Lehninger Principles of Biochemistry, Fifth Edition
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NADP is important for the erythrocyte in terms of requirement, since it is the major source of hydrogen atoms required to reduce the oxidised glutathione[15]. Reduced glutathione is necessary to repair the

effects of spontaneous oxidation of sulfhydryl groups which damage the RBC membrane.

Spontaneous oxidation of ferrous iron in hemeoccurseveryday. The product is methaemoglobin, containing iron in the ferric state. Methaemoglobin is converted back to haemoglobin by enzymatic reduction, using the NADH generated in the anaerobic glycolytic pathway and NADPH generated in the pentose phosphate pathway[16].

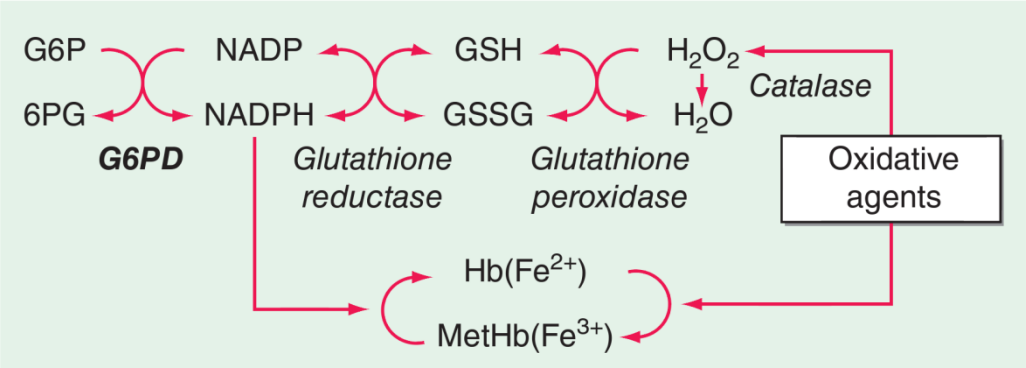


FIGURE 129-5 Diagram of redox metabolism in the red cell. 6PG, 6-phosphogluconate; G6P, glucose 6-phosphate; G6PD, glucose 6-phosphate dehydrogenase; GSH, reduced glutathione; GSSG, oxidized glutathione; Hb, hemoglobin; MetHb, methemoglobin; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate.

The summary of the metabolic processes taking place in the red blood cell is give in the following diagram[17].

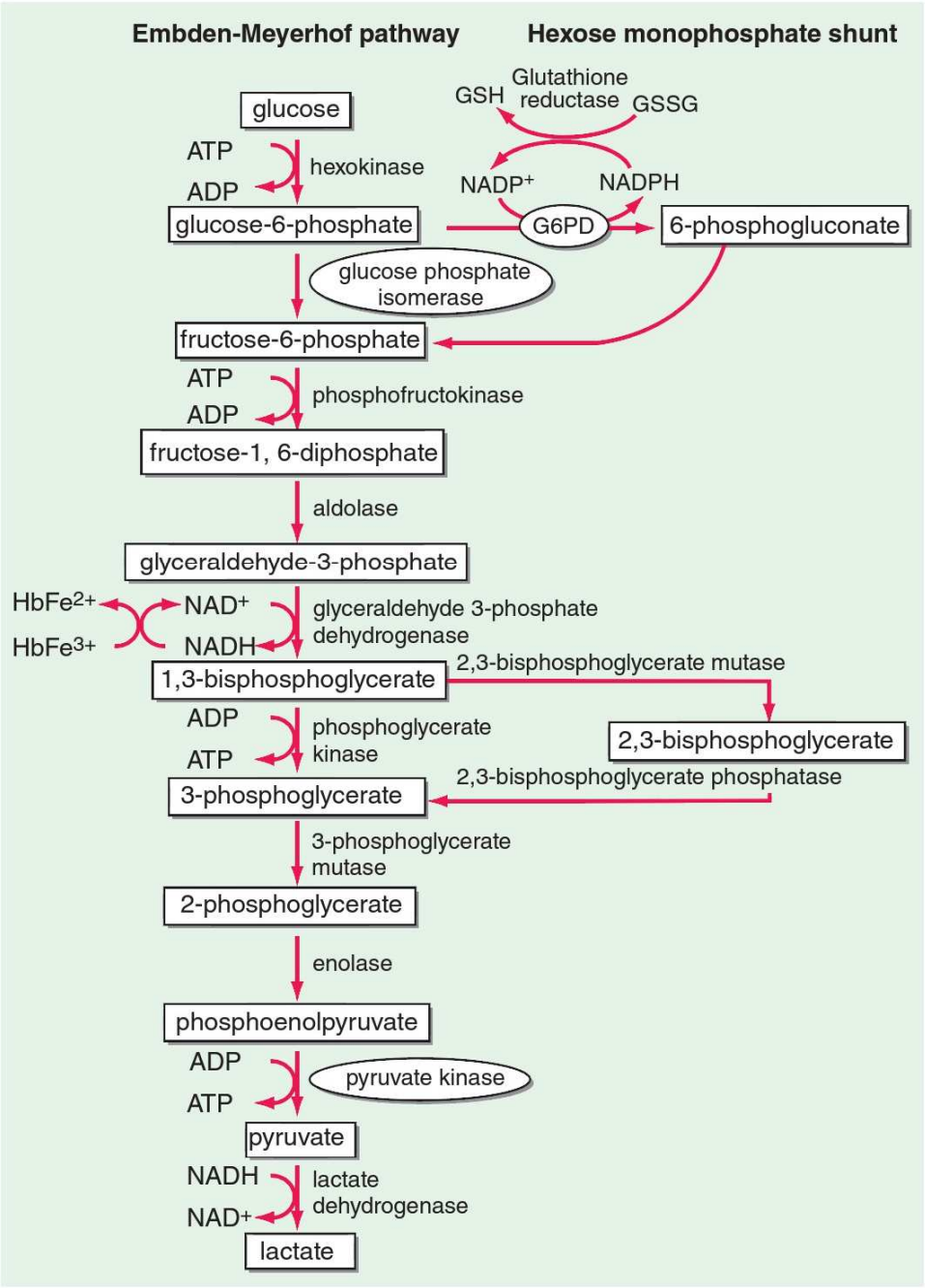


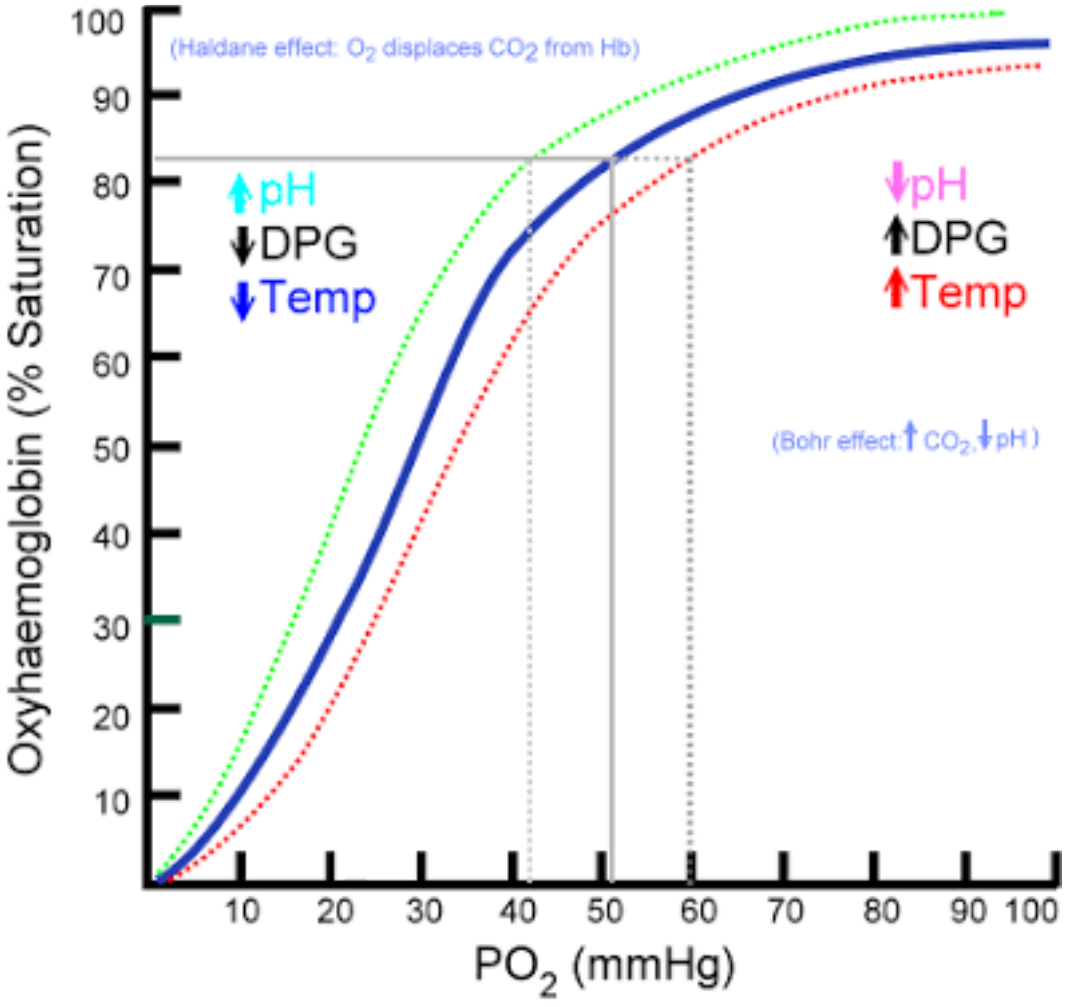
FIGURE 129-1 Red blood cell (RBC) metabolism. The Embden-Meyerhof pathway (glycolysis) generates ATP for energy and membrane maintenance. The generation of NADPH maintains hemoglobin in a reduced state. The hexose monophosphate shunt generates NADPH that is used to reduce glutathione, which protects the red cell against oxidant stress. Regulation of 2,3-bisphosphoglycerate levels is a critical determinant of oxygen affinity of hemoglobin. Enzyme deficiency states in order of prevalence: glucose 6-phosphate dehydrogenase (G6PD) > pyruvate kinase > glucose-6-phosphate isomerase > rare deficiencies of other enzymes in the pathway. The more common enzyme deficiencies are encircled.

FUNCTIONS OF THE RED BLOOD CELL:

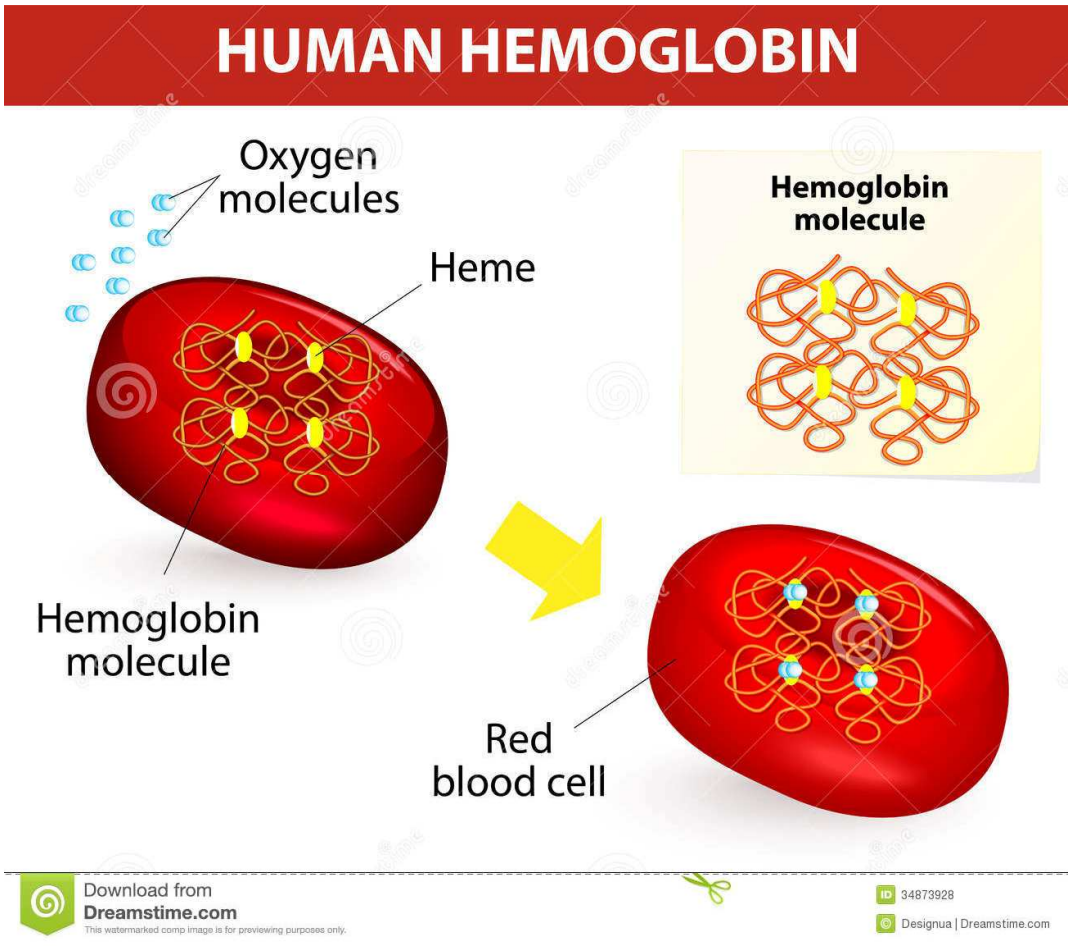
The presence of erythrocytes enables blood to carry about 100 times more oxygen than that could be transported by plasma alone.

Each gram of haemoglobin can carry 1.39 ml of oxygen, when it is fully saturated, the degree of saturation depending upon the oxygen tension (P_{O2}) of the blood. The degree of oxygen bound to haemoglobin is given by the oxygen dissociation curve[18].

The oxygen dissociation curve (ODC):



The ODC is sigmoidal in shape, thus implicating, the binding of oxygen to one heme molecule increases the binding of oxygen to other heme molecules. Thus the affinity for the last oxygen molecule is 100 times greater than the binding of the first oxygen molecule[19]. This phenomenon is called as the co-operative binding of oxygen to haemoglobin. The reverse also holds good, (i.e) release of one oxygen molecule from heme, facilitates the release of oxygen.



One molecule of heme binds with 4 molecules of oxygen.[20]

HEMOGLOBIN

Haemoglobin is a red blood cell pigment which is almost exclusively found in erythrocytes.

The normal concentration of Hb in blood is:

Males: 14-16 g/dl

Females: 12-14 g/dl[21]

The two major functions of haemoglobin are:

1. Delivers O_2 from lungs to tissues
2. Transports CO_2 and protons back from tissues to lungs

STRUCTURE OF HEMOGLOBIN:

Haemoglobin (mol. wt: 64, 450) is a tetrameric allosteric protein consisting of heme – the non protein part and globin – the apoprotein part[22].

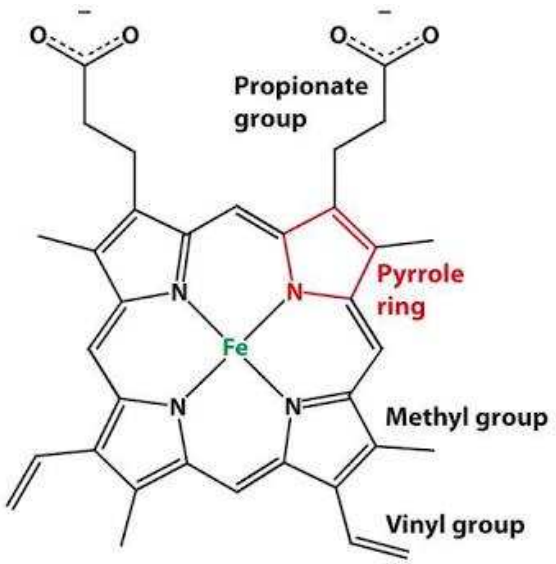
Structure of globin:

It consists of 4 polypeptide chains of two different primary structures. The common form is HbA_1 ($\alpha_2\beta_2$). The four subunits are held together by non-covalent interactions.

Form	Chain composition	Fraction of total hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbF	$\alpha_2\gamma_2$	<2%
HbA_2	$\alpha_2\delta_2$	2–5%
HbA_{1c}	$\alpha_2\beta_2$-glucose	3–6 %

Structure of heme:

It contains a porphyrin molecule (protoporphyrin IX) with iron at the centre to which the histidine group of the globin chain and one molecule of oxygen are attached, in a plane perpendicular to the pyrrole rings[23]. Protoporphyrin IX consists of 4 pyrrole rings to which 4 methyl, 2 propionyl and 2 vinyl groups are attached.



Heme
(Fe-protoporphyrin IX)

Unnumbered 7 p196
Biochemistry, Seventh Edition
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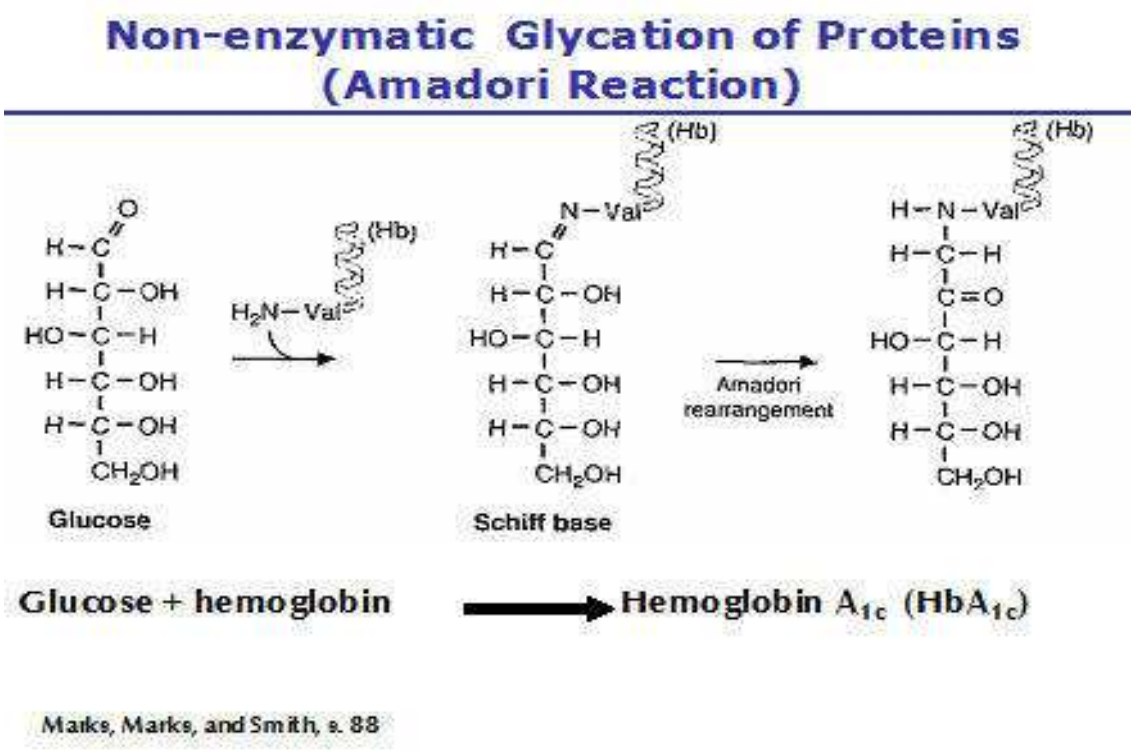
HbA_1c:

The rate of the glycation reaction is proportional to the hemoglobin concentration of the blood.And also, the accessibility of the side chain amino groups of hemoglobin for glucose is constant and so is the lifetime of the red blood cells. So, only the concentration of glucose should influence the concentration of HbA_1c[24]. Accordingly, HbA_1c would be a perfect proxy for blood glucose concentration over the lifespan of an erythrocyte.



Thus, HbA_1c is HbA_1,that is being glycated at the N-terminal amino group of the β chain of haemoglobin (βN-1-deoxyfructosyl-hemoglobin)[25].

The relationship between HbA_1c and plasma glucose level is complex. Many studies have shown that HbA_1c is an surrogate marker forthe mean plasma glucose over the preceding weeks to months.

Erythrocyte life span averages 120 days. The level of HbA_1c in the blood is contributed by all the circulating erythrocytes, from the oldest (120 days old) to the youngest. However, recent plasma glucose levels over the recent 3– 4 weeks earlier contribute more to the level of HbA_1c than does the long past plasma glucose levels (3–4 months earlier). Therefore, HbA_1c is just an average of blood glucose levels during the preceding 120 days[25].

Plasma glucose levels in the preceding 30 days contribute to about 50% to the final HbA_1c result, and plasma glucose levels from 90–120 days earlier contribute to just only 10% of the final value of HbA_1c [2]. This explains the increase or decrease in the level of HbA_1c with large changes in levels of blood glucose.

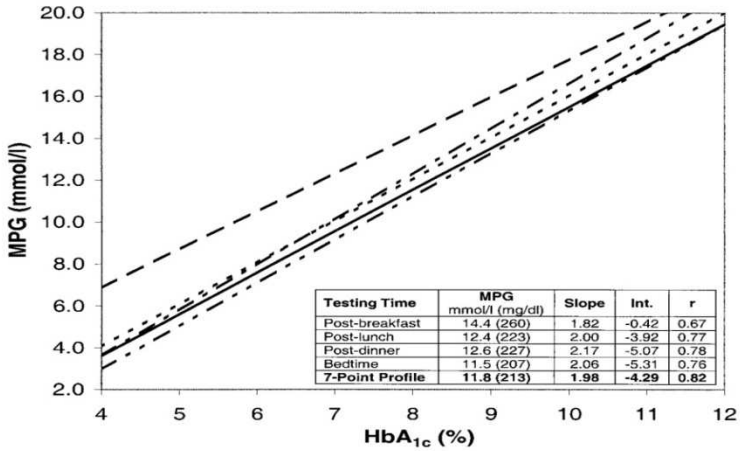


Figure 3— *Postmeal MPG and r at different testing times. — —, Postbreakfast; -----, Postlunch; — - —, postdinner; — - - —, bedtime; ——, seven-point.*

Thus, it is evident that it does not take 120 days to detect an appreciable change in HbA_1c after a change in mean plasma glucose.Thus HbA_1c is a marker for chronic hyperglycemia.

There is evidence that wide fluctuations can occur in HbA_1c levels between individuals which is not related to glycemic status of the individual, suggesting that there are “low glycators” and “high glycators”[7.8].

FPG when used alone, should be used with great caution as a measure of long-term glycemia. FPG tends to progressively underestimate HbA_1c (and seven-point MPG) at increasing levels of plasma glucose.

The data also suggest that the post-meal blood glucose contributes much to the HbA_1c levels; however, all post-meal times are not equal in their contribution.

Compared with the seven-point glucose profiles, post-breakfast blood glucose levels markedly overestimate the HbA_1c levels, whereas post-lunch glucose levels show a relationship withHbA_1c levels that is very much similar to that of mean plama glucose. A previous study also showed that in patients with type 2 diabetes, post-lunch plasma glucose levels is a better indicator of glycemic control than is FPG[9].

VARIOUS METHODS OF HbA_{1c} TESTING:

Advantages and disadvantages of various HbA1c assay methods

Assay	Principle	Advantages	Disadvantages
Ion Exchange Chromatography	HbA1c has lower isoelectric point and migrates faster than other Hb components.	Can inspect chromograms for Hb variants. Measurements with great precision.	Variable interference from hemoglobinopathies, HbF and carbamylated Hb but the current ion exchange assays correct for HbF and carbamylated Hb does not interfere.
Boronate Affinity	Glucose binds to m-aminophenylboronic acid.	Minimal interference from haemoglobinopathies, HbF and carbamylated Hb.	Measures not only glycation of N-terminal valine on β chain, but also β chains glycated at other sites and glycated α chains.
Immunoassays	Antibody binds to glucose and between 4-10 N-terminal amino acids on β chain.	Not affected by HbE, HbD or carbamylated Hb Relatively easy to implement under many different formats.	May be affected by haemoglobinopathies with altered amino acids on binding sites. Some interference with HbF.

RED CELL INDICES

The major red cell indices include:

1. Mean Corpuscular Volume (MCV)
2. Mean Corpuscular Hemoglobin (MCH)
3. Mean Corpuscular Hemoglobin Concentration (MCHC)
4. Red Cell Distribution Width (RDW)

Mean Corpuscular Volume (MCV):

It is an index of the volume of a single red blood cell. A subnormal MCV indicates microcytosis, while a supranormal MCV indicates macrocytosis.

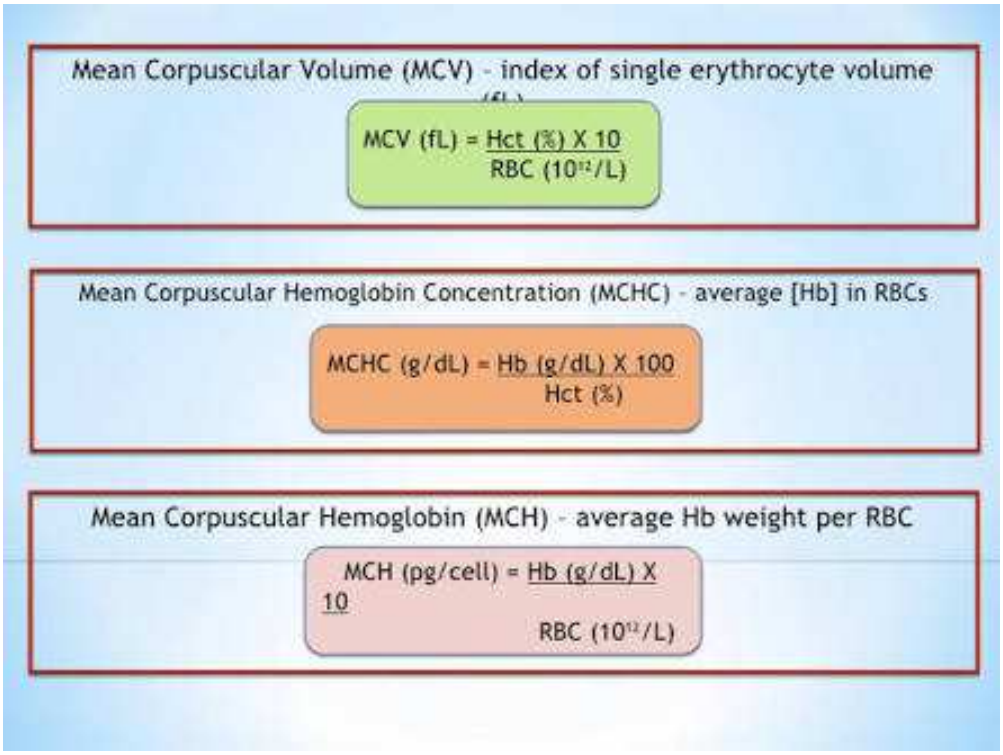
Mean Corpuscular Hemoglobin (MCH):

It is a measure of the amount of haemoglobin per red blood cell. A subnormal MCH denotes hypochromic RBCs.

Mean Corpuscular Hemoglobin Concentration (MCHC):

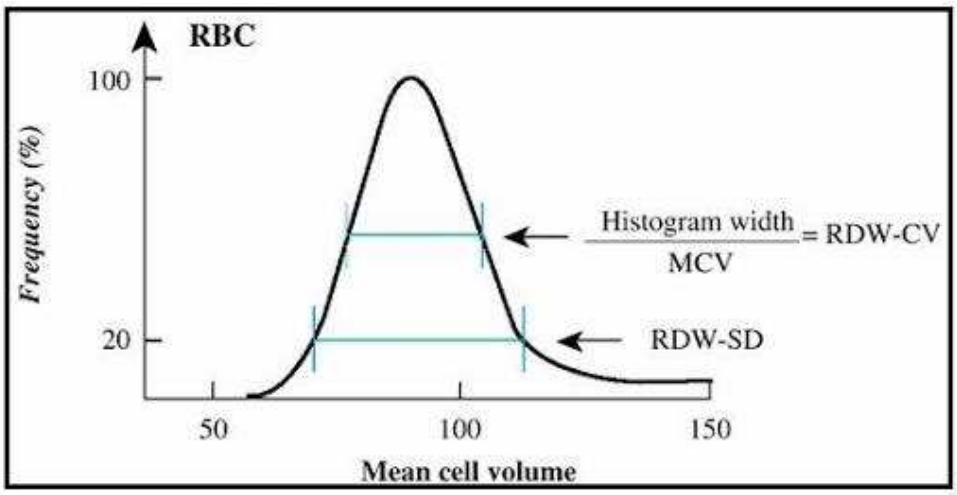
It measures the average amount of Hb in the erythrocytes, in toto. A subnormal MCHC indicates, interference in the synthesis of Hb. An elevated MCHC indicates, dehydration of the erythrocytes[26].

The calculation of these indices is given by the following formulas:



Red Cell Distribution Width (RDW):

It is a quantitative assessment of the variation in the red cell volume. It is increased in nutritional anemias like iron deficiency anemia and megaloblasticanemia, while it remains normal in cases of other causes of microcytic and macrocytic anemias.



It can be expressed in terms of Standard Deviation (RDW-SD), where the normal reference range is 39-46 fl, or as Co-efficient of Variation (RDW-CV), where the normal reference range is 11.6-14.0%.

The normal red cell indices are summarised below:

Normal adult red cell values.

	Male	Female
Haemoglobin (g/dL)	13.5–17.5	11.5–15.5
Haematocrit (PCV) (%)	40–52	36–48
Red cell count ($\times 10^{12}$/L)	4.5–6.5	3.9–5.6
Mean cell haemoglobin (MCH) (pg)	27–34	
Mean cell volume (MCV) (fL)	80–95	
Mean cell haemoglobin concentration (g/dL)	30–35	
Reticulocyte count ($\times 10^{9}$/L)	50–150	

PCV, packed cell volume.

ANEMIA

Anemiais present when the haemoglobin level or the hematocrit in the blood is below the lower reference range for age and sex of the individual, the lower limit of normalcy being reduced during pregnancy[22].

CLINICAL MANIFESTATIONS OF ANEMIA:

The common symptoms of anemia include:

- Fatigue
- Tiredness
- Effort intolerance
- Effort dyspnoea
- Palpitations
- Gidiness
- Effort angina

The most common sign of anemia is PALLOR. Pallor is usually absent if the haemoglobin level is more than 9 g/dl, and usually present if the haemoglobin level is less than 6 g/dl.

Other signs of anemia include high output failure and congestive cardiac failure in late stages.

TYPES OF ANEMIA:

Anemia can be classified based on:

- Morphology
- Pathophysiology

Morphological classification:

Anemia is classified morphologically based on MCV and MCH.

1. Microcytic Hypochromic Anemia:

   It is defined as MCV < 80 fl and MCH <27 pg.

   It includes the following disorders:

   - Iron deficiency anemia
   - Lead poisoning
   - Sideroblasticanemia

2. Normocytic Normochromic Anemia:

   It is defined by MCV 80-100 fl and MCH >27 pg

   It includes:

   - Haemolytic anemias
   - Anemia of chronic disease
   - Acute/Chronic blood loss
   - Renal disease

- Bone marrow failure (post-chemotherapy, infiltration by tumours, etc.,)

3. Macrocytic Anemia:

   It is defined by MCV >100 fl

   Some examples include the following:

   Megaloblastic:

   - Vit B_{12} deficiency
   - Folic acid deficiency

   Non – Megaloblastic:

   - Alcoholism
   - Liver disease
   - Myelodysplasia
   - Aplastic anemia

Pathophysiological classification:

1. Decreased production:

- Abnormal bone marrow
  - Aplastic anemia
    - ✓ Congenital
    - ✓ Acquired

- Pure red cell aplasia
    - ✓ Congenital (Diamond Blackfan)
    - ✓ Acquired
- Myelophthisis
    - ✓ Myelofibrosis
    - ✓ Leukemia
    - ✓ Cancer metastasis

- Essential factors deficiency
    - Deficiency anemia
        - ✓ Iron
        - ✓ Vit. B_{12}
        - ✓ Folic acid
    - Anemia in renal disease
        - ✓ Erythropoietin deficiency

- Stimulation factor deficiency
    - Anemia of chronic disease
    - Anemia of hypopituitarism
    - Anemia of hypothyroidism

- ineffective erythropoiesis
    - thalassemia
    - sideroblasticanemia

2. Increased destruction:

- Haemolytic anemia
    - Intra – corpuscular defect
        - ✓ Membrane defects
            - Spherocytosis
            - Ovalocytosis
            - Elliptocytosis
        - ✓ Enzyme defects
            - G-6-PD
            - Phosphokinase
        - ✓ Haemoglobin defects
            - Thalassemia
            - Hemoglobinopathies
    - Extra – corpuscular defect
        - ✓ Mechanical
            - MAHA
        - ✓ Chemical & Physical
        - ✓ Infection
        - ✓ Antibodies
        - ✓ Hyperspleenism

- Blood loss
    - Acute blood loss
        - ✓ Trauma
        - ✓ GI bleeding
    - Chronic blood loss
        - ✓ Hypermenorrhoea
        - ✓ Parasitic infections

Importance of blood film examination:

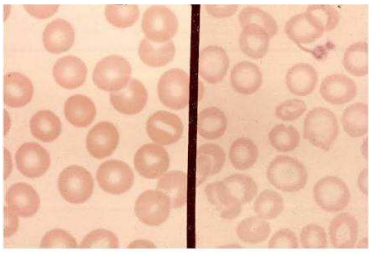
- Low power view: information regarding type and number of leucocytes
- High power view:
    - Dry lens: deviation of red cell morphology, atypical cells
    - Oil immersion lens: finer details of abnormal red blood cells and leucocytes

Key features to be noted on smear examination regarding erythrocyte are:

- Red cell size and shape
- Red cell chromasia
- Inclusions
- Abnormal/Atypical cells
- Reticulocytes

MICROCYTIC ANEMIA

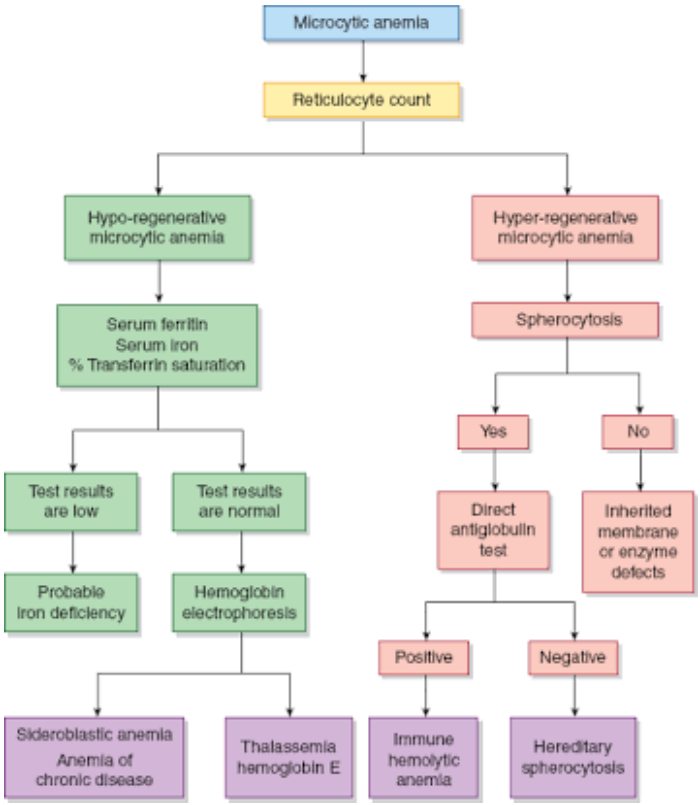
Microcytic anemia is characterised by the presence of small RBCs on a peripheral smear study and MCV < 80 fl on laboratory studies.



Normal red blood cells Microcytic anemia

There is increased central pallor (i.e) more than 1/3rd of the diameter of the RBC

The diagnostic algorithm of microcytic anemia is as given below:



DIABETES MELLITUS

Diabetes mellitus (DM) is a metabolic disorder characterised by the presence of hyperglycemia[27]. Raised glucose levels in the blood may speed up the process of atherosclerosis through various mechanisms such as oxidative stress and protein glycation of vessel walls, discussed in detail below.

Complications of diabetes mellitus:

Long term diabetes would lead to complications. They can be grouped into two types, as microvascular and macrovascular[28].

1. Microvascular complications:
    - Neuropathy
        - Autonomic
        - Sensory and motor
    - Nephropathy
    - Ocular
        - Macular edema
        - Retinopathy
2. Macrovacular complications:

    Coronary artery disease

    Cerebrovascular disease

    Peripheral arterial disease

PATHOGENESIS OF COMPLICATIONS:

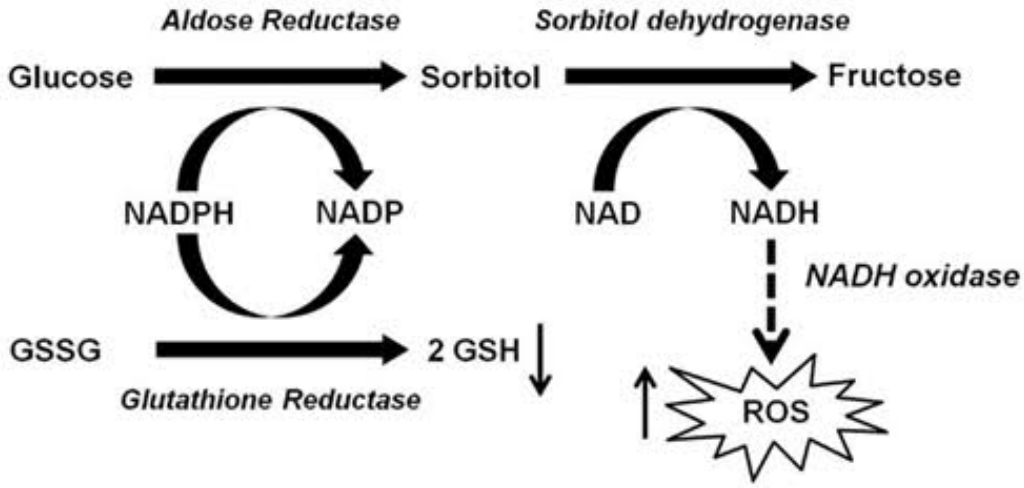
The pathogenic factors can be classified into two major categories namely, vascular and metabolic changes[29].

1. **Vascular changes:**
    - Reduced blood vessel contractility
    - Increased vascular permeability
    - Thickened basement membrane
    - Endothelial dysfunction
    - Increased factor VII, vWF& PGI
    - Increased fibrinogen, CRP, PAI-I
    - Neovascularisation
2. **Metabolic changes:**
    - Aldose reductase activity (polyol-sorbitol pathway)
    - Diacyl glycerol – protein kinase C activation (myoinositol pathway)[30]
    - Formation of advanced glycation end products (AGE)
    - Formation of reactive oxygen species

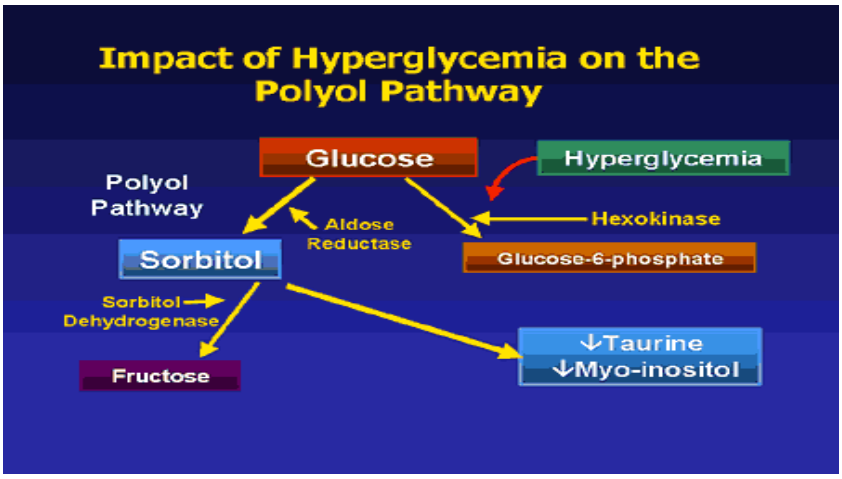
Of all the mechanisms listed above, advanced glycation end products have been proved to be most important mechanism bearing more implications and is a novel target of therapy and will continue in the future[31].

A. POLYOL – SORBITOL PATHWAY:

Glucose gets converted to sorbitol (polyol) by the action of aldose reductase which is later converted to fructose by the enzyme sorbitol dehydrogenase. The second step requires conversion of NAD to NADH resulting in the generation of reactive oxygen species, thus contributing to the pathogenesis of diabetes[32].



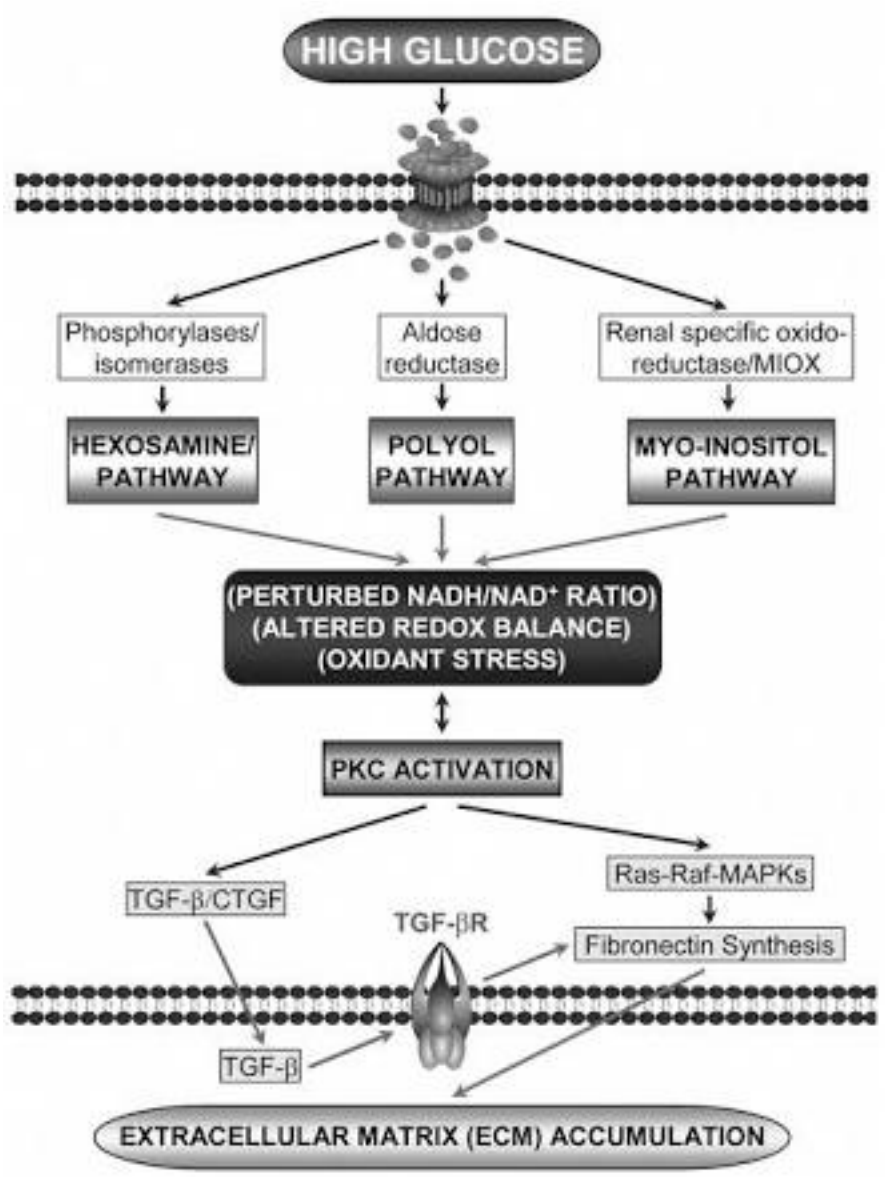
Conversion of sorbitol to fructose also results in the decrease inmyo-inositol, which later leads to activation of the protein kinase C (PKC) pathway.



B. PROTEIN KINASE C PATHWAY:

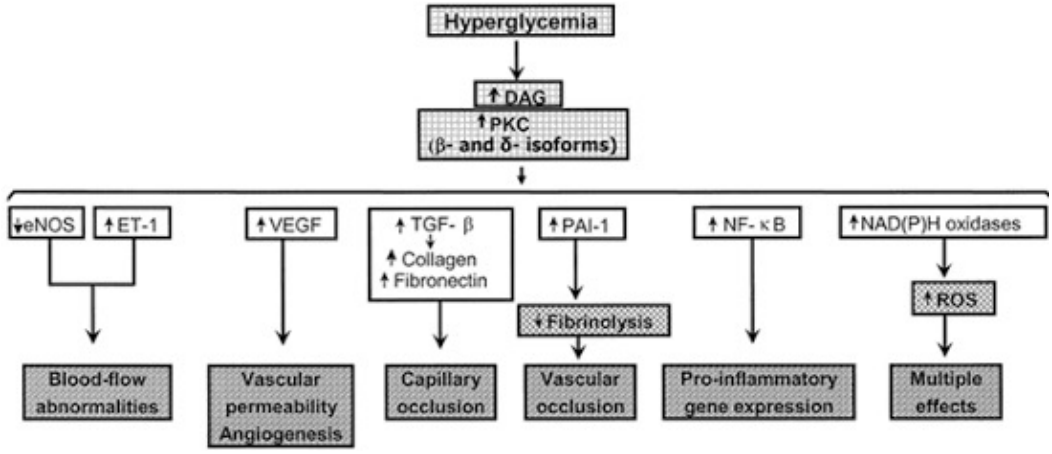
Reduced myoinositol and chronic hyperglycemia per se, stimulate protein kinase C pathway resulting in:

- Increased vascular permeability
- Increased vasoactive hormones
- Basement membrane thickening[33]



Reduced myo-inositol affects the Na^{+}-K^{+} ATPase pump, particularly in neurons, resulting in the accumulation of intacellular sodium. Thus the conduction velocity of the neurons are reduced, causing various forms of neuropathy ranging from autonomic to sensori – motor.

Myo-inositol deficiency has also been implicated in the pathogenesis of retinopathy and cataracts[34].

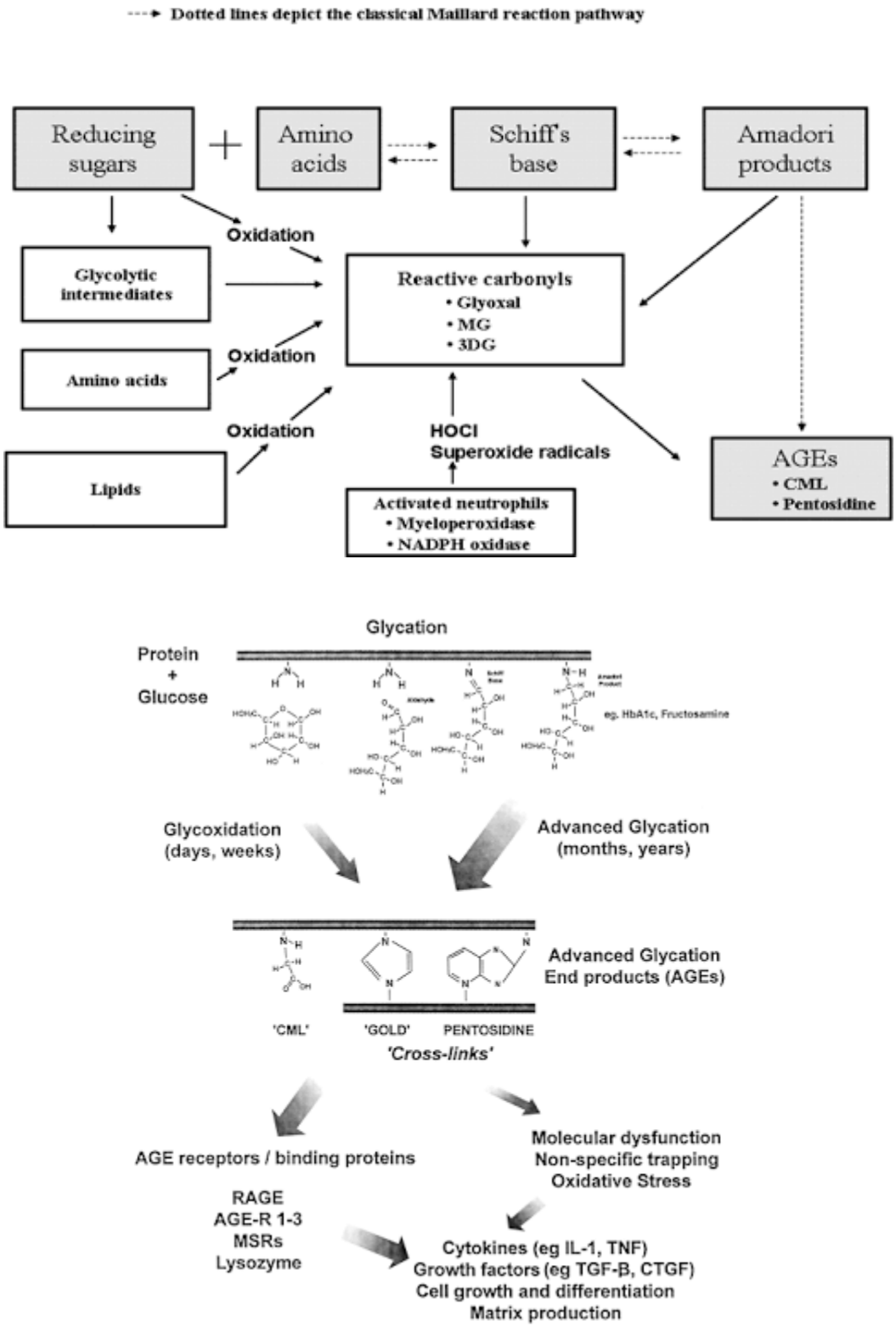


C. ADVANCED GLYCATION END PRODUCTS (AGE):

Glycation is a chemical process involving modification of proteins with reduing sugars, thus indicating a possible association between hyperglcemia and a wide variety of tissue pathologies.

Glucose forms a chemically reversible product with the proteins called as Schiff's base. The reducing sugars then react with the amino groups of the long lived proteins to produce non – enzymatic cross links. Formation of these cross-links occur at the end of the Maillard

reaction and those end products are called as Advanced Glycation End – products (AGE)[35].



AGEs are a group of complex, unstable, reactive compounds formed in excess in states of hyperglycemia. They alter the structural properties of tissue proteins and thereby reducing their susceptibility to catabolism[36].

The quantity of these AGEs can be used as surrogate markers for predicting the complications of diabetes, for example the blood levels of HbA_1c correlate with the development of retinopathy.

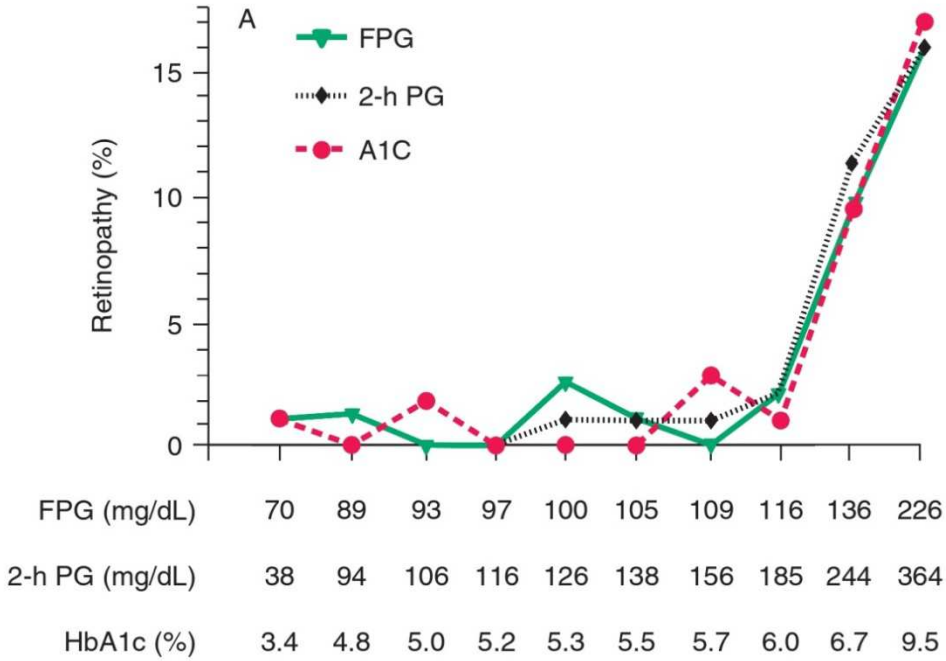
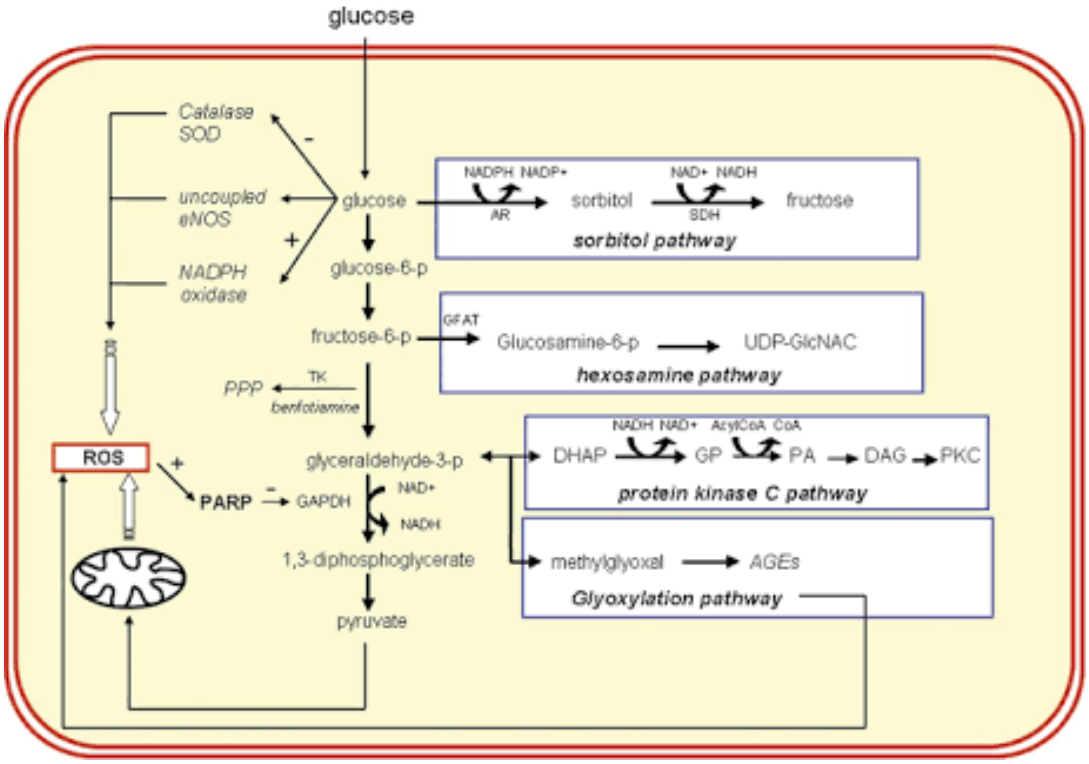


FIGURE 417-3 Relationship of diabetes-specific complication and glucose tolerance. This figure shows the incidence of retinopathy in Pima Indians as a function of the fasting plasma glucose (FPG), the 2-h plasma glucose after a 75-g oral glucose challenge (2-h PG), or the hemoglobin A_{1c} (HbA_{1c}). Note that the incidence of retinopathy greatly increases at a fasting plasma glucose >116 mg/dL, a 2-h plasma glucose of 185 mg/dL, or an HbA_{1c} >6.5%. (Blood glucose values are shown in mg/dL; to convert to mmol/L, divide value by 18.) *(Copyright 2002, American Diabetes Association. From Diabetes Care 25[Suppl 1]: S5–S20, 2002.)*

The summary of the pathophysiological changes taking place due to hyperglycemia is shown below[37].



All the mechanisms result in the production of reactive oxygen species and thus mitochondrial injury at the cellular level, thus resulting in tissue changes.

High glucose produces super oxide anion from the endothelial cells, which may quench nitric oxide, a potent endothelial derived vasodilator. Oxidative stress also interferes with endothelial dependant relaxation and cell replication, all of which culminating in the vascular complications of diabetes mellitus.

SCREENING OF DIABETES MELLITUS

Current recommendations suggest screening for diabetes mellitus in:

- Asymptomatic men >45 yrs
- Asymptomatic women >55 yrs
- The screening of the Pacific and the Indo – Asian population should begin for: Men at 35 yrs

  Women at 45 yrs[38]

Screening should be done once in 3 – 5 yrs depending on the risk.

Screening for diabetes should begin at 25 yrs of age in people with the following risk factors:

- Ischemic heart disease
- Cerebro-vascular disease
- Peripheral arterial disease
- Long term treatment with steroids
- Long term anti – psychotic use
- BMI ≥ 30 (≥27 for Indo – Asian population)
- Family history oftype 2 diabetes at an early age of onset in more than one first degree relative
- Past personal history of GDM

Additional risk factors include:

- Central obesity
- Impaired glucose tolerance on previous assessment
- Adverse lipid profile
- High blood pressure
- Polycystic ovary syndrome (PCOS)
- Current smoker (or) have quit smoking within the last 12 months

Children and young adults with BMI ≥ 30 (≥27 for the Indo – Asian population) should be screened for diabetes if:

- Family history of type 2 diabetes at an early age of onset
- Pacific or Indo – Asian ethinicity[48]

HbA_1c plays an important role in the screening of diabetes mellitus. So it is prudent that HbA_1c levels measured in any person should be sufficiently standardised and care has to be taken that no false positives or false negatives can be encouraged.

DIAGNOSIS OF DIABETES MELLITUS

ADA recommended criteria for the diagnosis of diabetes mellitus is as follows[61]:

TABLE 1. Criteria for the Diagnosis of Diabetes

FPG ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hours.*
OR
2-hour plasma glucose ≥200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*
OR
A1C ≥6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*
OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).**

**In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.*

***Only diagnostic in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis.*

Abnormal glucose homeostasis is defined as:

(1) FPG of 100–125 mg/dL is defined as impaired fasting glucose (IFG)

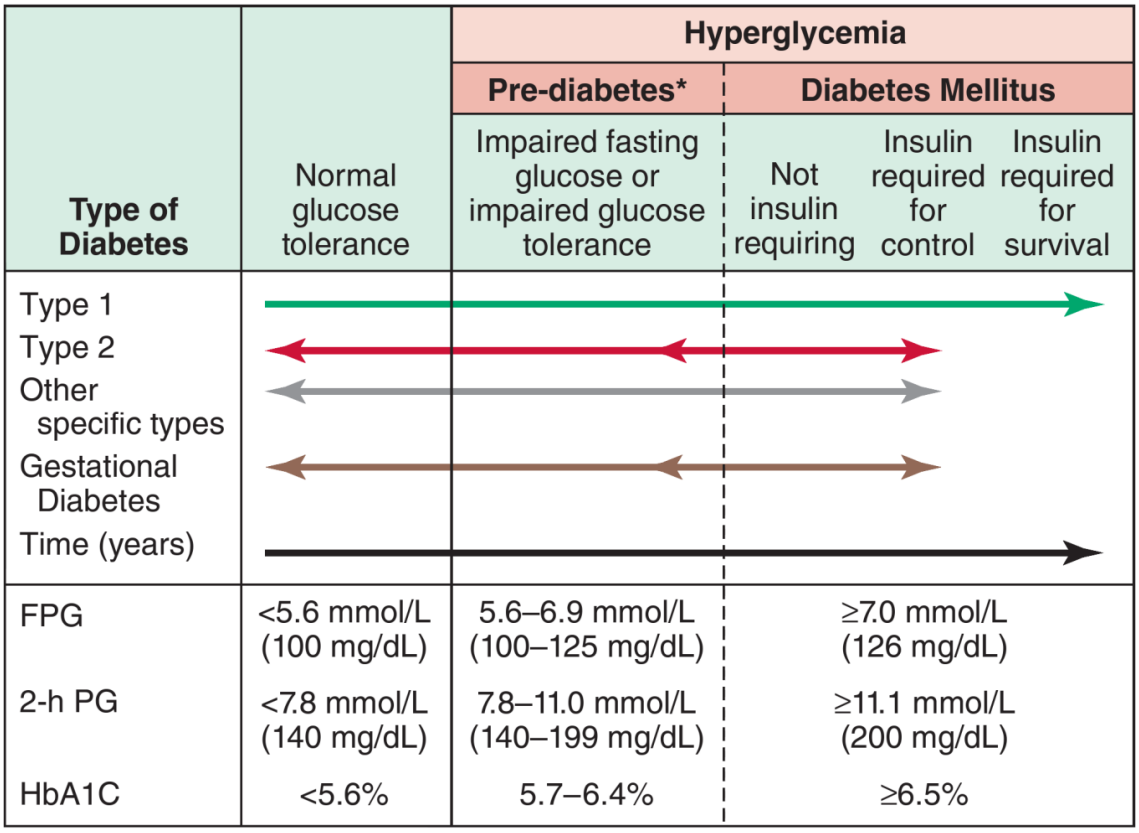
(2) plasma glucose levels between 140 and 199 mg/dL following an oral glucose challenge is termed as impaired glucose tolerance (IGT)

(3) HbA_1c of 5.7–6.4%.

An HbA_1c of 5.7–6.4%, IFG, and IGT are not equivalents, but instead, it denotes the individuals in all the three categories who are at a greater risk of progressing to type 2 DM and diabetes – related complications.People in these groups should be counseled about ways to decrease these risks[40].

The current criteria for the diagnosis of DM emphasizes the fact that either **HbA_1c or the FPG** as the most reliable tests for identifying DM in asymptomatic individuals[41].

The following picture shows the spectrum of normoglycemia and hyperglycemia:



ROLE OF HbA_1c IN DIABETES

The results of the Diabetes Control and Complications Trial (DCCT) which was published in 1993, and the U.K. Prospective Diabetes Study, published in 1998 showed that the relationship between HbA_1c levels and the risk for development of diabetic complications in patients with type 1 and type 2 diabetes, respectively.

In patients with diabetes, reducing plasma HbA_1c levels by glycaemic control would lower the risk ofmicrovascular and macrovascular disease.

Patients without diabetes, but with sub-optimalHbA_1c levels may still have a level of dysglycaemia that may not yet meet the diagnostic criteria of diabetes mellitus, but may yet still contribute to an adverse outcome. Plasma HbA_1c would be a more useful test to identify such patients, who are at risk.

Studies have shown that, increase in HbA_1c of 1% corresponds to a 20–30% increase in mortality or cardiovascular events.Thus, HbA_1c resembles blood pressure or cholesterol in terms of the continuous relation with cardiovascular risk[42].

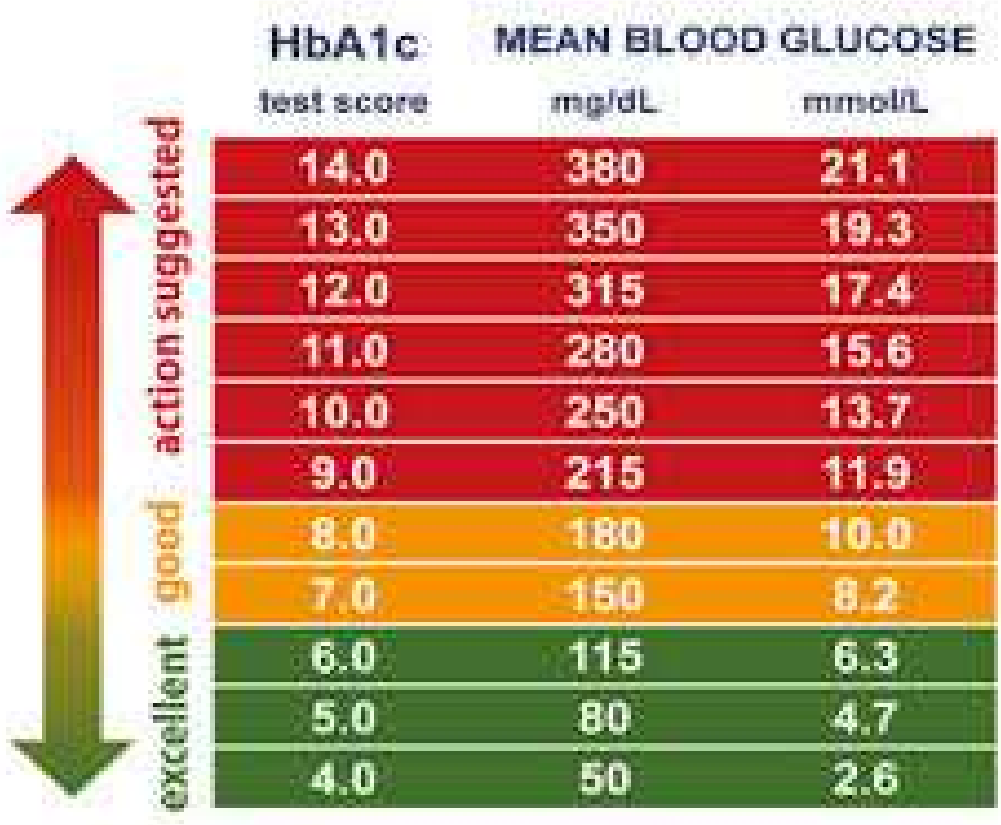
Protein glycation includes haemoglobin, plasma proteins, membrane proteins, lens proteins, etc., of which glycosylated HbA_1c forms the major fraction (80%).

HbA_1c serves as a reliable indicator of diabetes control over the past 90 days, effectiveness of treatment and risk of development of acute or long term complications.

Normal HbA_1c levels and their interpretation:

Non-diabetic range	4.5-5.8%
Risk of hypoglycaemia	<4.5%
Diabetic range	>6.5%
Pre-diabetic range	5.8-6.5%

Approximate correlation between levels and mean plasma glucose levels:



ADVANTAGES OF HbA_1c:

- No need of fasting
- Day to day variations in plasma glucose values is less
- Less biological variability associated with HbA_1c
- Established relationship between HbA_1c and the future risk of retinopathy
- Simpler sampling and analysis requirements[43]

FPG AND HbA_1c :

Fasting plasma glucose is still considered a valid test for the diagnosis of people with type 2 diabetes, when HbA_1c levels cannot be used. FPG can also be used when there is a discrepancy between two values of HbA_1c. In such situations FPG would prove useful to clarify the diagnosis.

Thus, FPG is the preferred as the initial test if the patient has a specific condition or a complication that is most likely to alter the HbA_1c value. The criteria for diagnosis of diabetes with FPG and OGTT remains unchanged though.

The following table depicts the difference in FPG and HbA_1c:

Table 1. Advantages and disadvantages of HbA_{1c} and fasting glucose assays.[4,5,17]

	Fasting glucose	HbA_{1c}
Patient preparation	Fasting required, this is often misunderstood or not adhered to	None
Sample processing	Stringent requirements for processing and separation; rarely achieved	Relatively simple
Standardisation	Fully standardised	Fully standardised
Variability	Moderate pre-analytic and biological variation	Little to no variation
Effect of illness	Severe illness may increase glucose concentration in hours or days	Severe illness may shorten red-cell lifespan, reducing HbA_{1c} levels in days or weeks
Haemoglobinopathies and disorders of red blood cell turnover	Few problems	May interfere with values in some cases
Cost to laboratory (approximate)	$2.30	$11.40

Thus HbA_1c has advantages over FPG, except in terms of cost, which is the limiting factor for using HbA_1c in developing countries.

Another point of interest is values of FPG and HbA_1c during illness. Any illness, as a response by the body, increases the blood sugar levels, thereby increasing the values of FPG. Whereas, the life span of the RBC is reduced in illness, thereby reducing the values of HbA_1c. Other than this, the values of FPG and HbA_1c go hand in hand to each other[44].

The following table gives a comparision between the glucose assys and HbA_1c:

	Glucose assays	**HbA_1c**
Patient preparation prior to collection of blood	Stringent requirements	None
Processing of blood	Separation and storage of plasma or serum sample minimally at 4^0C	Avoid conditions for > 12 hrs at temperatures $>23^0C$. otherwise keep the sample at 4^0C
Measurement	Widely available all over the world	Not widely available all over the world
Standardization	Standardised to reference procedures	Standardised to reference procedures
Routine calibration	Adequately done	Adequately done
Illness	Increasess the concentration of blood glucose	Shorten lifespan of RBC, and thus reduces HbA_1c
Hemoglobinopathies	Little problem unless the patient is ill	Interferes in the measurement
Affordability	Affordable in most low and middle income countries	Not affordable in most low and middle income countries

FACTORS INFLUENCING THE MEASUREMENT OF HbA_1c LEVELS:

1. Erythropoiesis:

- Increased HbA_1c:
    - iron deficiency
    - vitamin B_{12} deficiency
    - decreased erythropoiesis
- Decreased HbA_1c:
    - administration of erythropoietin
    - iron supplementation
    - vitamin B12 supplementation
    - reticulocytosis
    - chronic liver disease

2. Altered Haemoglobin structure:

- Genetic or chemical alterations in haemoglobin structure:
    - haemoglobinopathies
    - HbF
    - Methaemoglobin (variable HbA_1c)

3. Glycation of HbA_1c:

- Increased HbA_1c:
    - Alcoholism
    - chronic renal failure
    - decrease in intra – erythrocyte pH
- Decreased HbA_1c:
    - aspirin
    - vitamin C and E
    - certainhaemoglobinopathies
    - increase in intra – erythrocyte pH
- Variable HbA_1c:
    - genetic determinants

4. Erythrocyte destruction:

- Increased HbA_1c:
    - increased erythrocyte life span: Spleenectomy
- Decreased HbA_1c:
    - decreased life span of the erythrocyte:
        - haemoglobinopathies
        - spleenomegaly
        - rheumatoid arthritis

- drugs
    - ✓ antiretrovirals
    - ✓ ribavirin
    - ✓ dapsone

5. HbA_1cAssays:

- Increased HbA_1c:
    - Hyperbilirubinaemia
    - carbamylated haemoglobin
    - alcoholism
    - large doses of aspirin
    - chronic opiate use
- Decreased HbA_1c:
    - hypertriglyceridaemia
- Variable HbA_1c:
    - haemoglobinopathies

Thus, false elevation of HbA_1c levels are encountered when there is an increase in the RBC lifespan and any condition that shortens the lifespan of RBC is likely to decrease the HbA_1c levels[46].

MATERIALS AND METHODS

MATERIALS AND METHODS

The study was conducted in the Department of Internal Medicine, Madras Medical College, Rajiv Gandhi Government General Hospital, Chennai – 600003.

ETHICAL COMMITTEE APPROVAL:

Obtained.

PATIENT CONSENT:

Obtained.

DURATION OF THE STUDY:

6 months.

STUDY DESIGN:

Case control study.

SAMPLE SIZE:

100 cases.

100 controls.

INCLUSION CRITERIA:

Confirmed cases of microcyticanemia as evidenced by:

1. Hb< 12 g/dl (women); < 13 g/dl (men).
2. MCV < 80 fl.
3. Peripheral smear showing microcytosis.

EXCLUSION CRITERIA:

1. Acute/Chronic blood loss
2. Hemolytic anemia
3. Hemoglobinopathies
4. Chronic kidney disease
5. Pregnancy
6. Established diabetes
7. Impaired fasting glucose
8. Impaired glucose tolerance
9. Family H/O diabetes
10. Obesity

SELECTION OF CASES:

Patients in the general medical ward meeting the inclusion and exclusion criteria were selected for the study.

CONTROLS:

Age and sex matched subjects who did not have microcytic anemia but still meeting the exclusion criteria were selected as subjects.

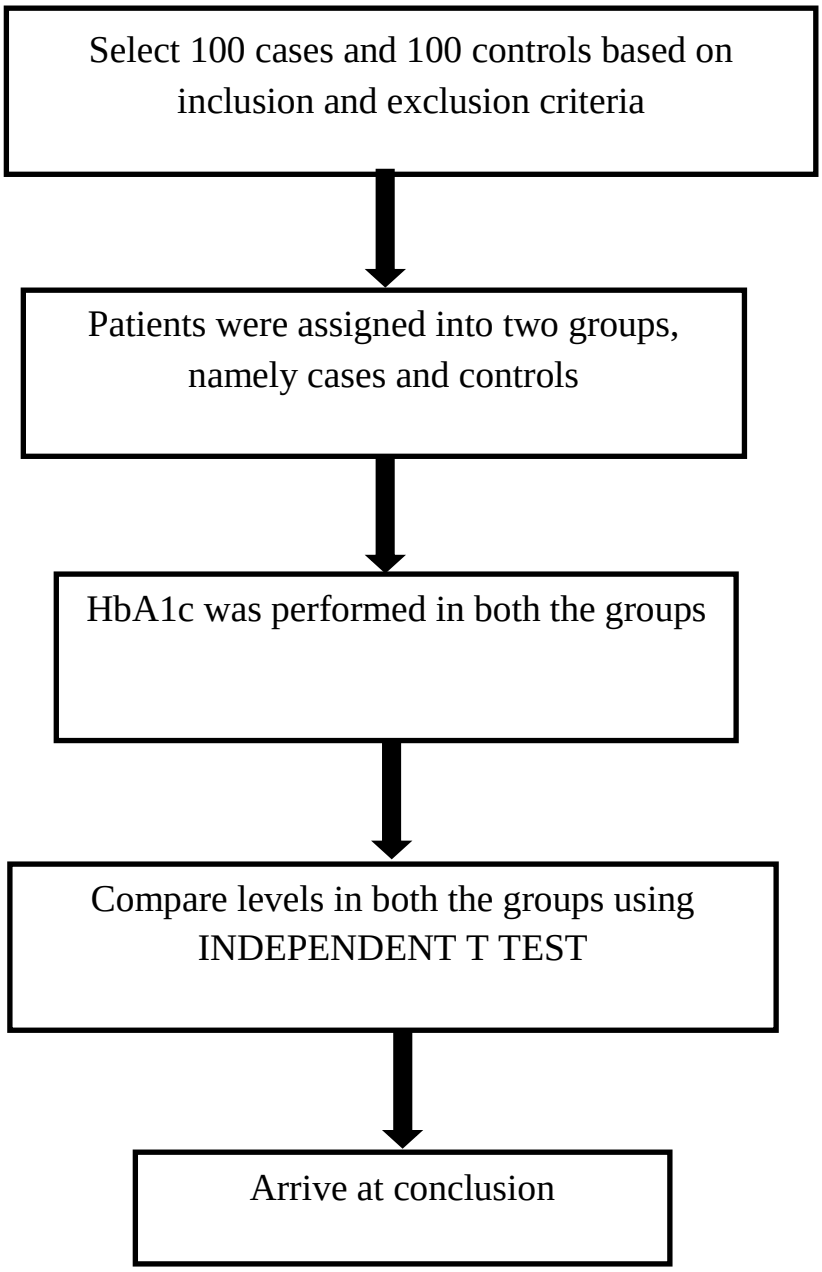
DATA COLLECTION AND METHODS:

A total of 100 cases and 100 controls from general medical ward/OPD are selected according to inclusion and exclusion criteria and patients were subjected to following investigations:

1. Complete hemogram
2. Peripheral smear study
3. Fasting blood glucose (FBG)
4. Post Prandial Blood Glucose (PPBG)
5. Oral Glucose Tolerance Test (OGTT)
6. Glycosylated hemoglobin (HbA_1c)

HbA_1c levels was then compared between both the groups and its correlation with microcytic anemia was calculated.

METHODOLOGY:



STATISTICAL ANALYSIS:

Data was entered in Microsoft Excel spread sheet and analysed using the software-Epidemiological Information Package 2002 (Epi-INFO 2002)-developed by the centre of disease control and prevention, Atlanta for World Health Organisation. Range, Median, Mean, Standard Deviation and ‘p’ values were calculated using this package. Chi-square test was done to find out the significance of relationship between the groups. Statistical significance was considered if the ‘p’ value was below 0.05.

SPONSORSHIP:

No.

CONFLICT OF INTEREST:

None.

OBSERVATIONS AND RESULTS

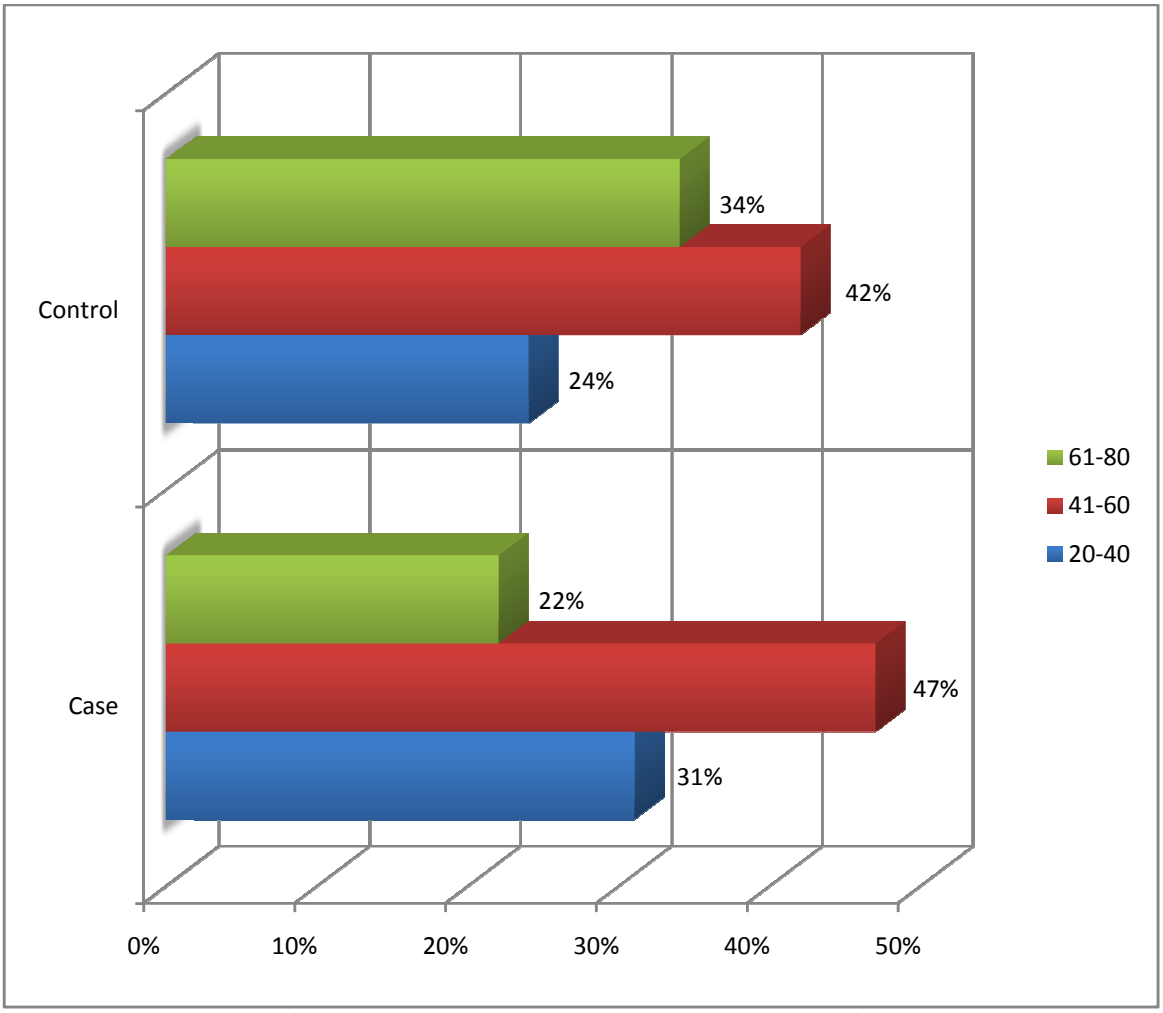
OBSERVATION AND RESULTS

AGE WISE DISTRIBUTION OF CASES AND CONTROLS

			Age Group			Total
			20-40	41-60	61-80	
Group	Case	Count	31	47	22	100
		% within group	31.0%	47.0%	22.0%	100.0%
	Control	Count	24	42	34	100
		% within group	24.0%	42.0%	34.0%	100.0%
Total		Count	55	89	56	200
		% within group	27.5%	44.5%	28.0%	100.0%

Cases and controls were selected between age of 20 – 80 yrs. 47% of the cases and 42% of the controls fell within the age group of 40 – 60 yrs.

BAR DIAGRAM DEPICTING THE AGE DISTRIBUTION OF CASES AND CONTROLS

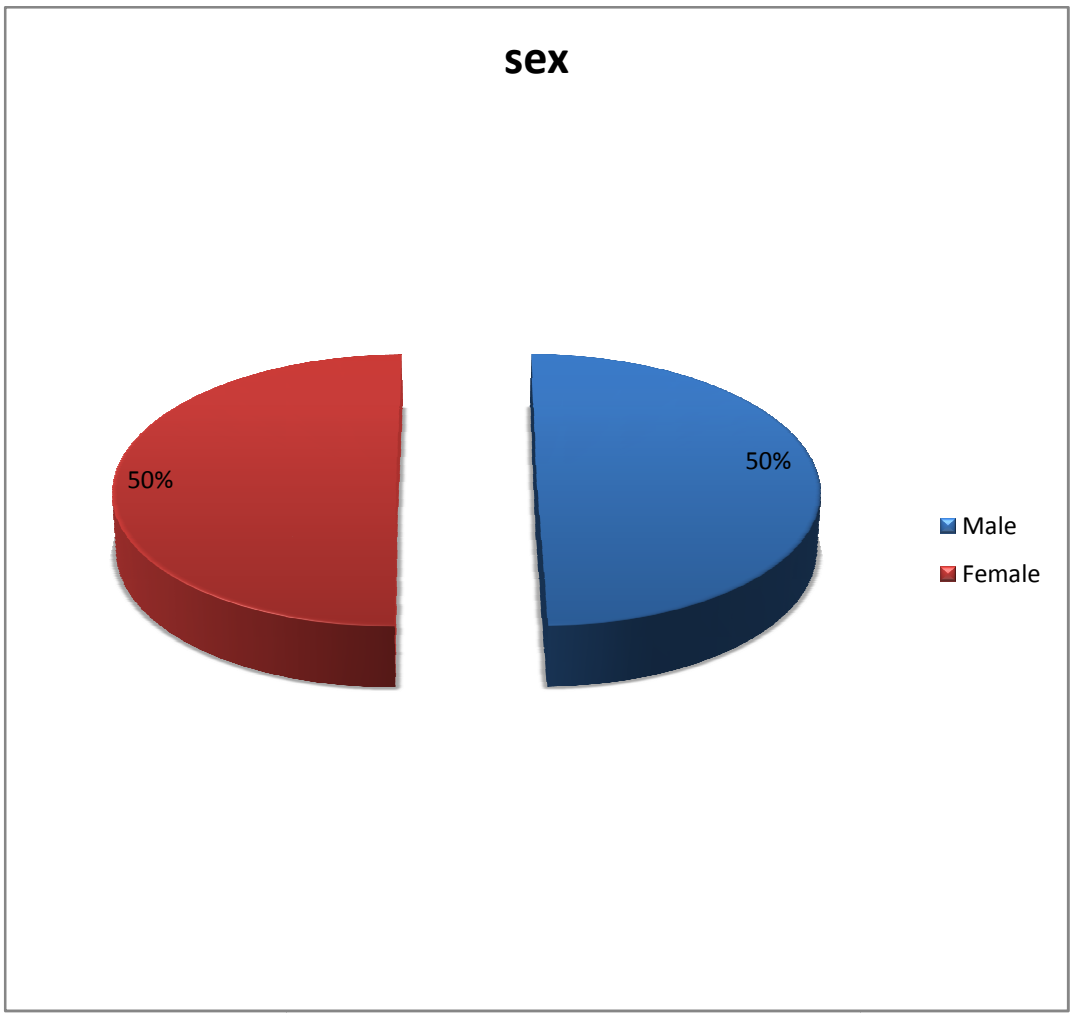


SEX DISTRIBUTION OF CASES AND CONTROLS

			SEX		Total
			Male	Female	
Group	Case	Count	49	51	100
		% within SEX	49.5%	50.5%	50.0%
	Control	Count	50	50	100
		% within SEX	50.5%	49.5%	50.0%
Total		Count	99	101	200
		% within SEX	100.0%	100.0%	100.0%

The sex distribution between the case and controls were almost equal. 49.5% of the cases were males and 50.5% were females. 50.5% of the controls were males and 49.5% were females.

BAR DIAGRAM DEPICTING THE SEX DISTRIBUTION OF CASES AND CONTROLS



CASE AND CONTROL DEFINITION

			Hb Group		Total
			Normal	Anemic	
Group	Case	Count	0	100	100
		% within Hb Group	0.0%	100.0%	50.0%
	Control	Count	100	0	100
		% within Hb Group	100.0%	0.0%	50.0%
Total		Count	100	100	200
		% within Hb Group	100.0%	100.0%	100.0%

Cases were anemic patients, defined by Hb< 12 g/dl (for females) and < 13 g/dl (for males). Controls were non – anemic subjects.

CORRELATION BETWEEN MCV AND HbA_1c

			HbA_1c Group		Total
			<6.5 Normal	>6.5 Abnormal	
MCV Group	50-60	Count	0	6	6
		% within HbA1c Group	0.0%	7.0%	3.0%
	61-70	Count	9	38	47
		% within HbA1c Group	7.9%	44.2%	23.5%
	71-80	Count	6	40	46
		% within HbA1c Group	5.3%	46.5%	23.0%
	81-90	Count	70	1	71
		% within HbA1c Group	61.4%	1.2%	35.5%
	above 90	Count	29	1	30
		% within HbA1c Group	25.4%	1.2%	15.0%
Total		Count	114	86	200
		% within HbA1c Group	100.0%	100.0%	100.0%

Pearson Chi-Square=141.058*p<0.001

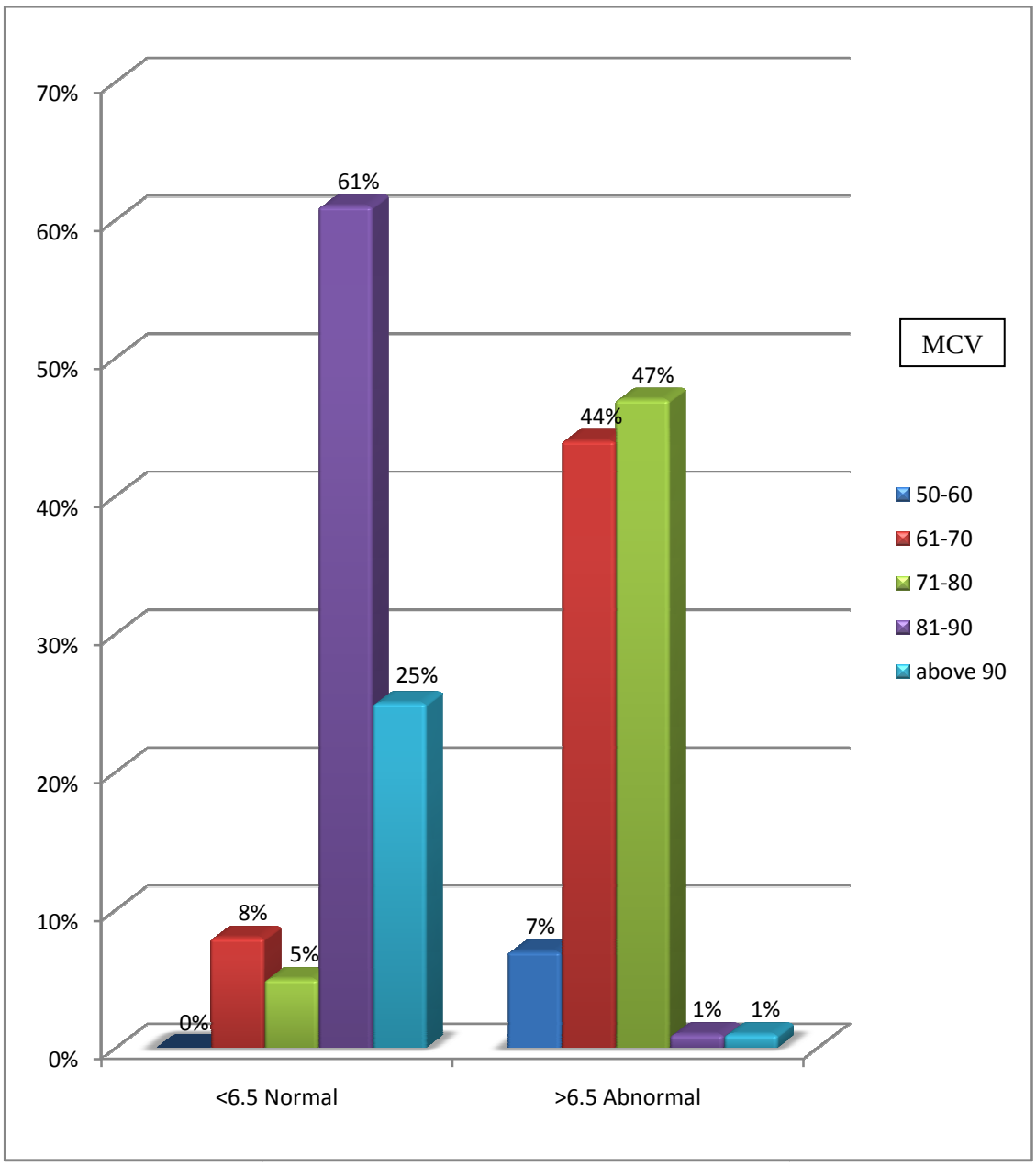
ANALYSIS OF MCV VALUES AND HbA_1c

44.2% of the patients with MCV values ranging between 61 – 70 fl had an abnormal HbA_1c values > 6.5% and 46.5% of the patients with MCV values ranging between 71 – 80 fl had HbA_1c values > 6.5%.

61.4% of the patients with MCV values ranging between 81 – 90 fl had HbA_1c values < 6.5% and 25.4% of the patients with MCV values above 90 fl had a normal HbA_1c values < 6.5%.

The effect of MCV values on HbA_1c were analysed using the independent t test. **The 'p' value obtained was <0.001 which is statistically significant**. Thus proving, the lower the values of MCV below 80 fl, more the tendency for abnormal HbA_1c (>6.5%).

BAR DIAGRAM DEPICTING THE CORRELATION BETWEEN MCV AND HbA_1c



CORRELATION BETWEEN Hb AND MCV

			Hb Group		Total
			>12 Normal	<12 Anemic	
MCV Group	50-60	Count	0	6	6
		% within Hb Group	0.0%	6.0%	3.0%
	61-70	Count	0	47	47
		% within Hb Group	0.0%	47.0%	23.5%
	71-80	Count	0	46	46
		% within Hb Group	0.0%	46.0%	23.0%
	81-90	Count	70	1	71
		% within Hb Group	70.0%	1.0%	35.5%
	above 90	Count	30	0	30
		% within Hb Group	30.0%	0.0%	15.0%
Total		Count	100	100	200
		% within Hb Group	100.0%	100.0%	100.0%

Pearson Chi-Square=196.056*p<0.001

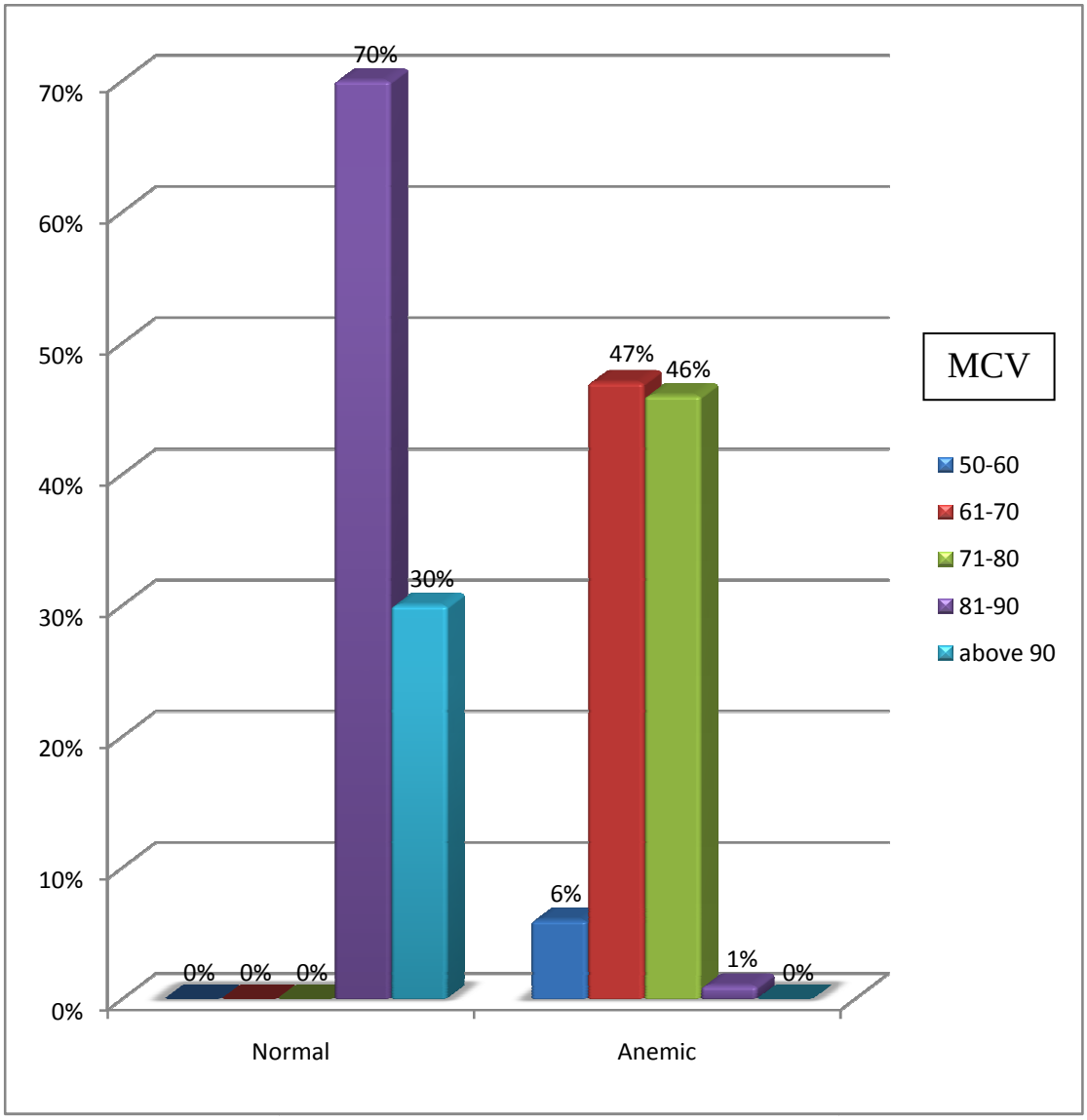
ANALYSIS OF MCV VALUES AND Hb

47% of the patients with MCV values between 61 – 70 fl were anaemic (as defined by the criteria as mentioned above) and 46% of the patients with MCV values between 71 – 80 fl were anaemic.

70% of the patients with MCV values between 81 – 90 fl were normal and 30% of the patients with MCV values above 90 fl were normal.

The effect of MCV values on Hb were analysed using the independent t test. **The 'p' value obtained was <0.001 which is statistically significant**. Thus proving, the lower the values of MCV below 80 fl, more the tendency for anemia (i.e) low Hb.

BAR DIAGRAM DEPICTING THE CORRELATION BETWEEN MCV AND Hb



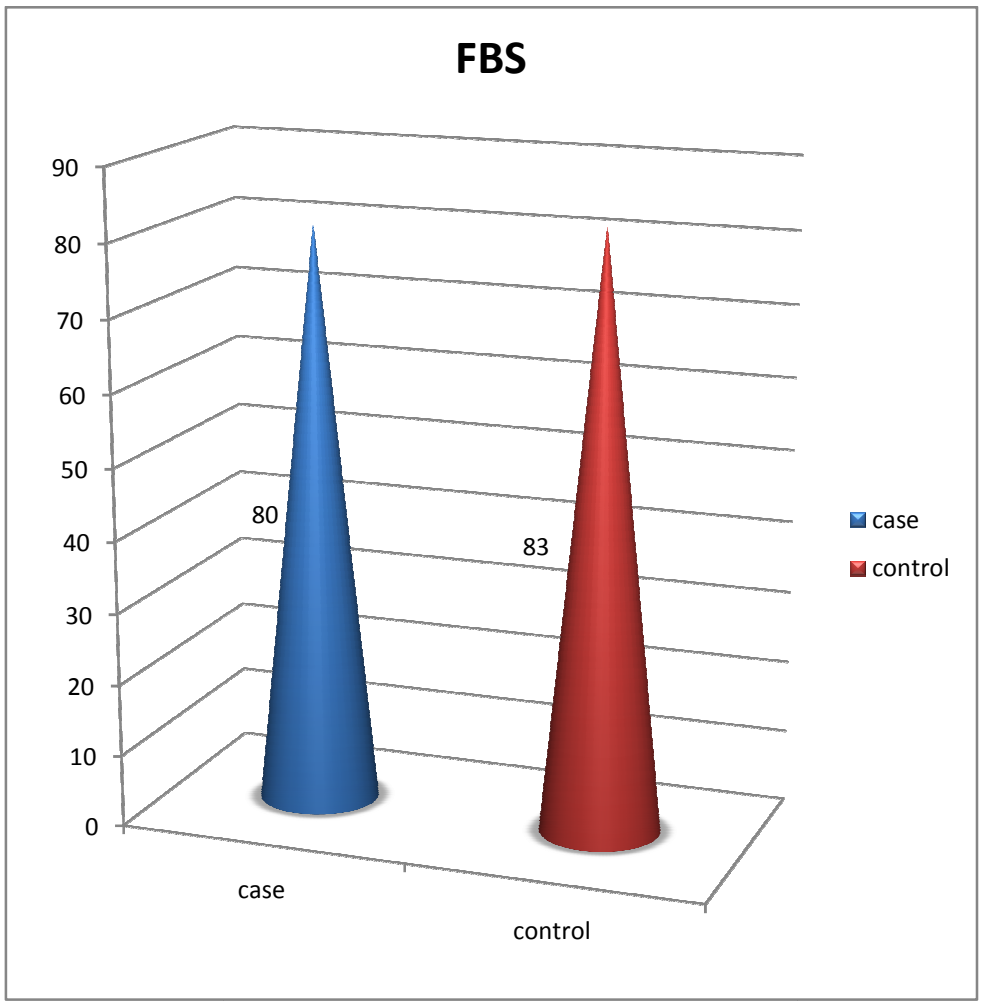
GROUP STATISTICS

	Group	N	Mean	Std. Deviation	Std. Error Mean
FBS	**Case**	100	80.4000	9.30081	.93008
	Control	100	82.5200	8.07475	.80748
PPBS	**Case**	100	116.6600	9.45721	.94572
	Control	100	120.5100	11.45081	1.14508
OGTT (2hrs)	**Case**	100	114.7500	8.92208	.89221
	Control	100	120.2200	11.59727	1.15973

BAR DIAGRAM DEPICTING THE MEAN FBS

The FBS of the cases was 80.40 ± 9.30 mg/dl.

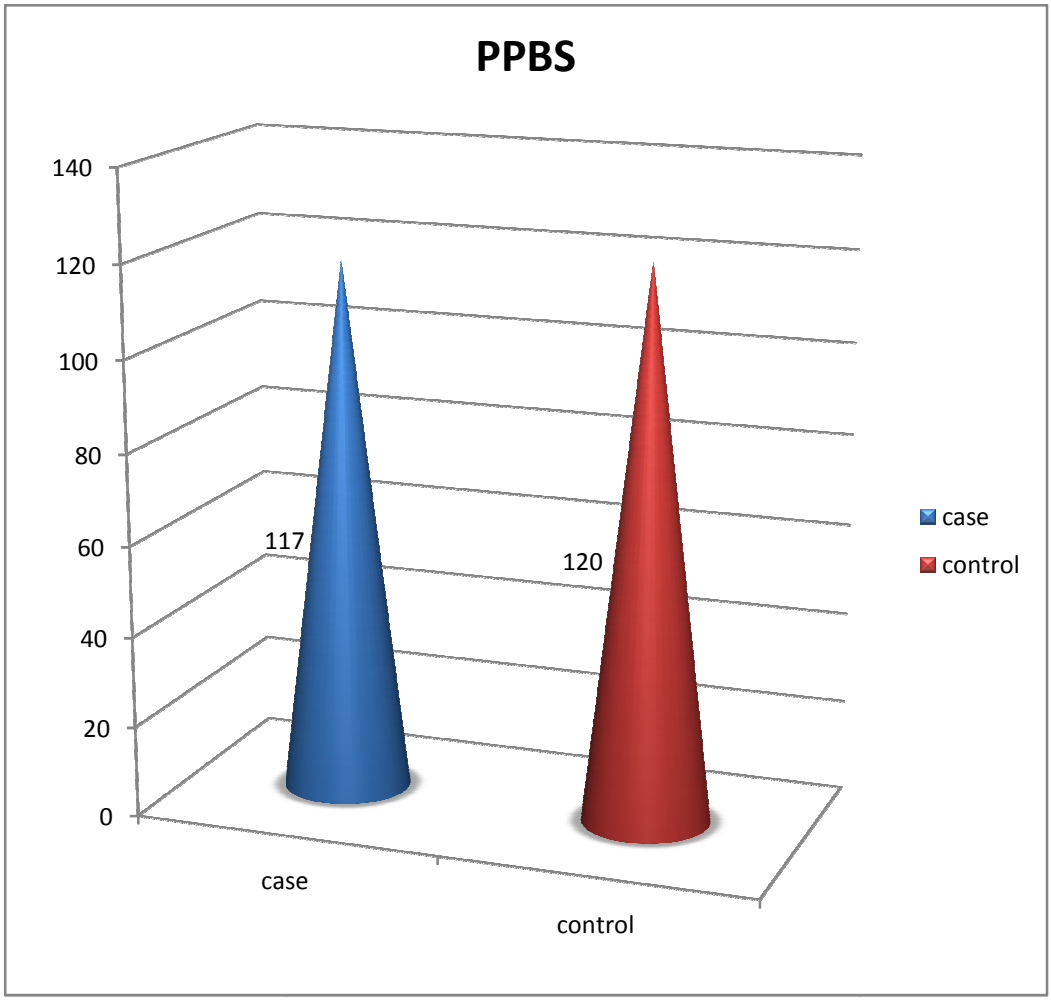
The FBS of the controls was 82.52 ± 8.07 mg/dl.



BAR DIAGRAM DEPICTING THE MEAN PPBS

The PPBS of the cases was 116.66 ± 9.45 mg/dl.

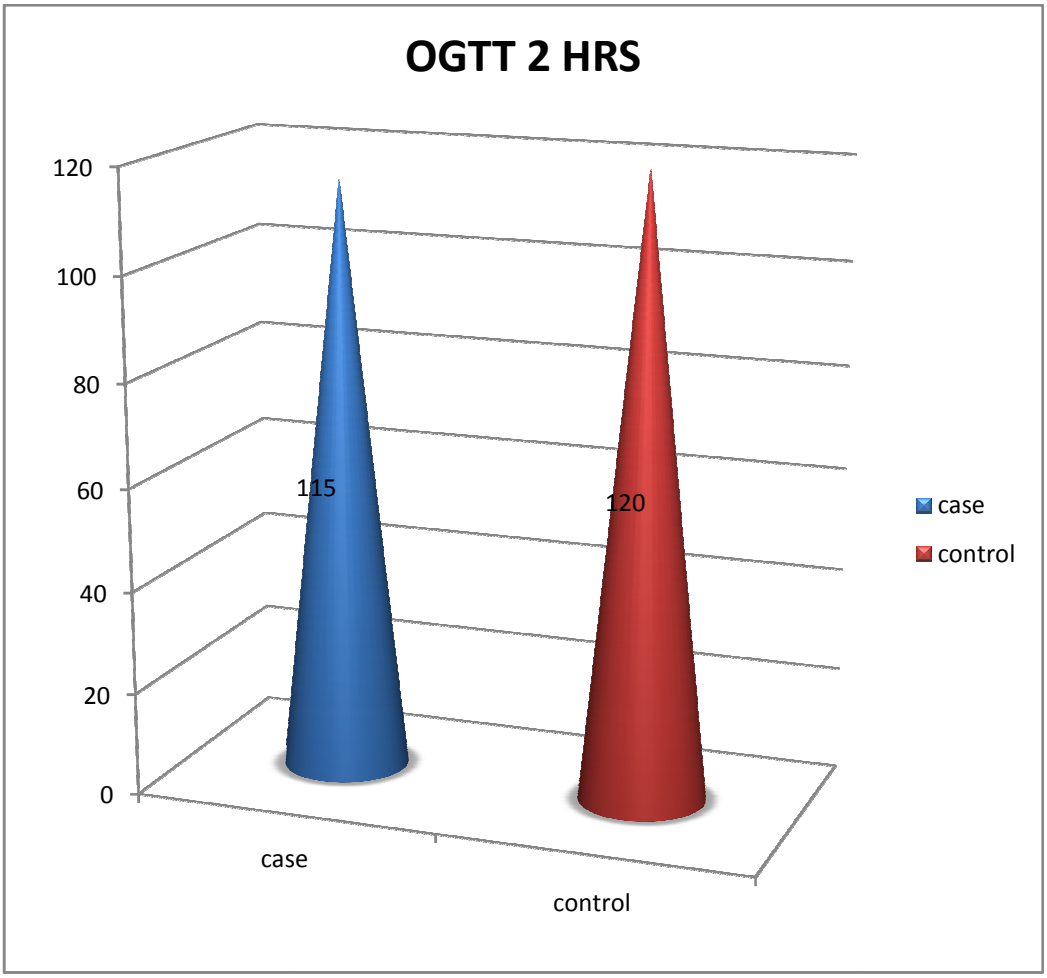
The PPBS of the controls was 120.51 ± 11.45 mg/dl.



BAR DIAGRAM DEPICTING MEAN OGTT (2 hrs)

The OGTT (2hrs) of the cases was 114.75 ± 8.92 mg/dl.

The OGTT (2hrs) of the controls was 120.22 ± 11.59 mg/dl.



GROUP STATISTICS

	Group	N	Mean	Std. Deviation	Std. Error Mean	Calculated t-Statistic
Hb	**Case**	100	10.0780	1.29985	.12999	21.728*
	Control	100	14.2570	1.41880	.14188	
MCV	**Case**	100	70.6930	5.85236	.58524	23.895*
	Control	100	88.4560	4.58353	.45835	
HbA1c	**Case**	100	6.8950	.55748	.05575	24.573*
	Control	100	5.3560	.28544	.02854	

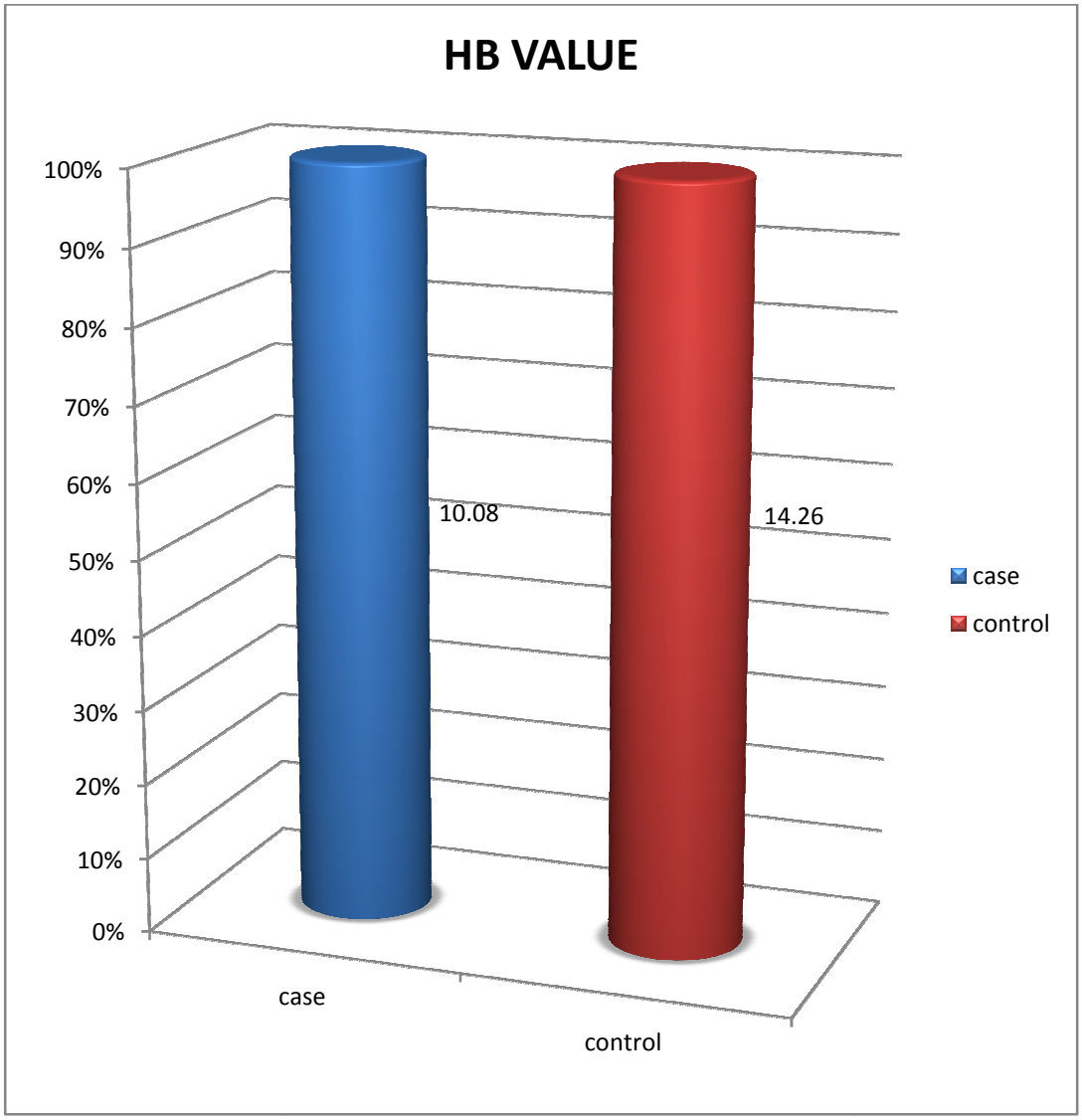
***p<0.001**

The 'p' value of the calculated t-statistic was <0.001, which is statistically significant

BAR DIAGRAM DEPICTING THE MEAN Hb

The Hb of the cases was 10.07 ± 1.29 g/dl with a calculated t-statistic of 21.728

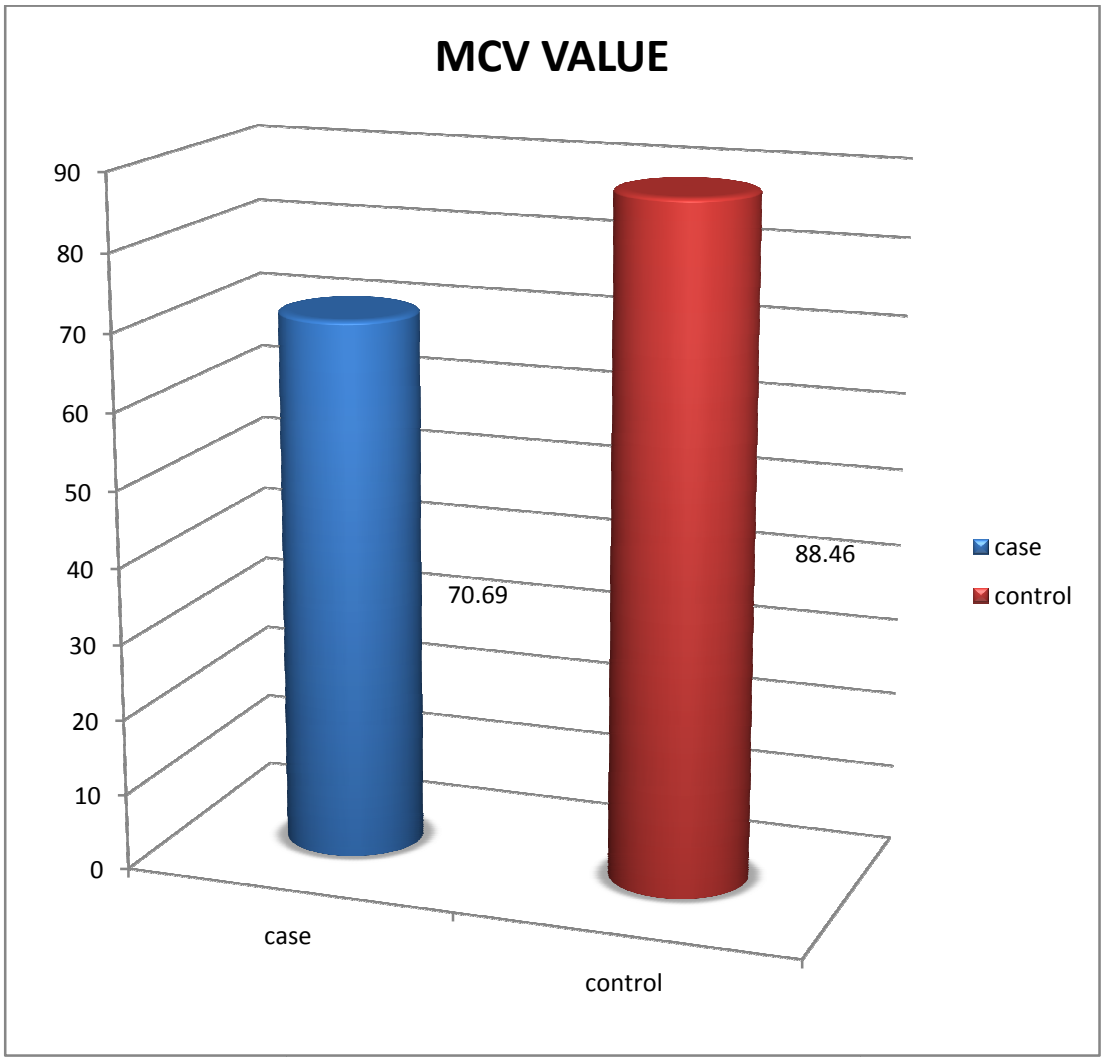
The Hb of the controls was 14.25 ± 1.41 g/dl with a calculated t-statistic of 21.728



BAR DIAGRAM DEPICTING THE MEAN MCV

The MCV of the cases was 70.69 ± 5.85 fl with a calculated t-statistic of 23.895

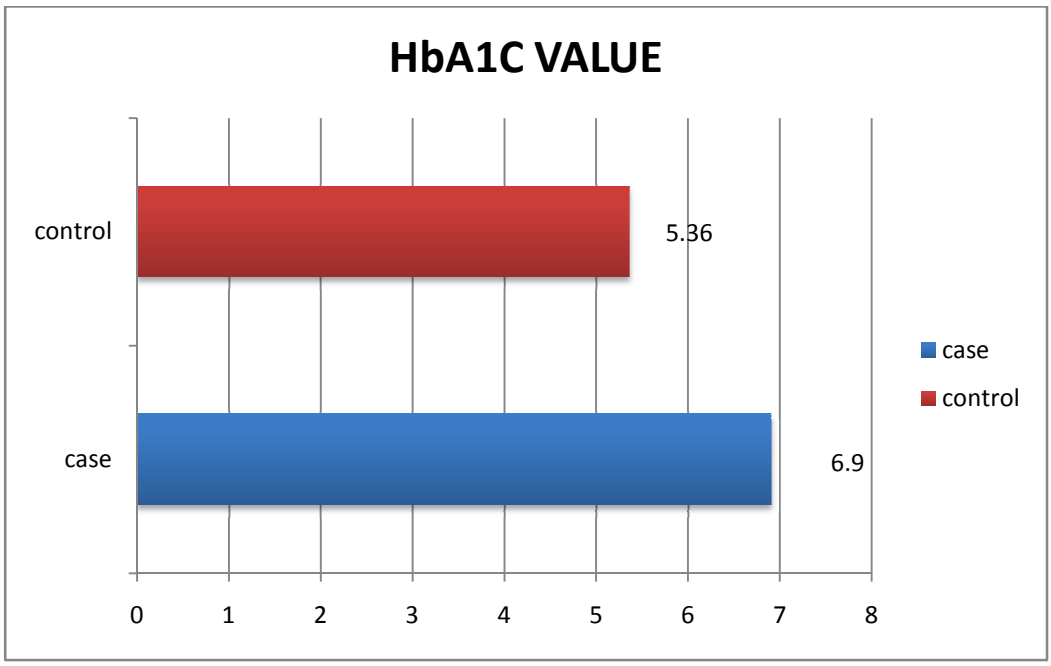
The MCV of the controls was 88.45 ± 4.58 fl with a calculated t-statistic of 23.895



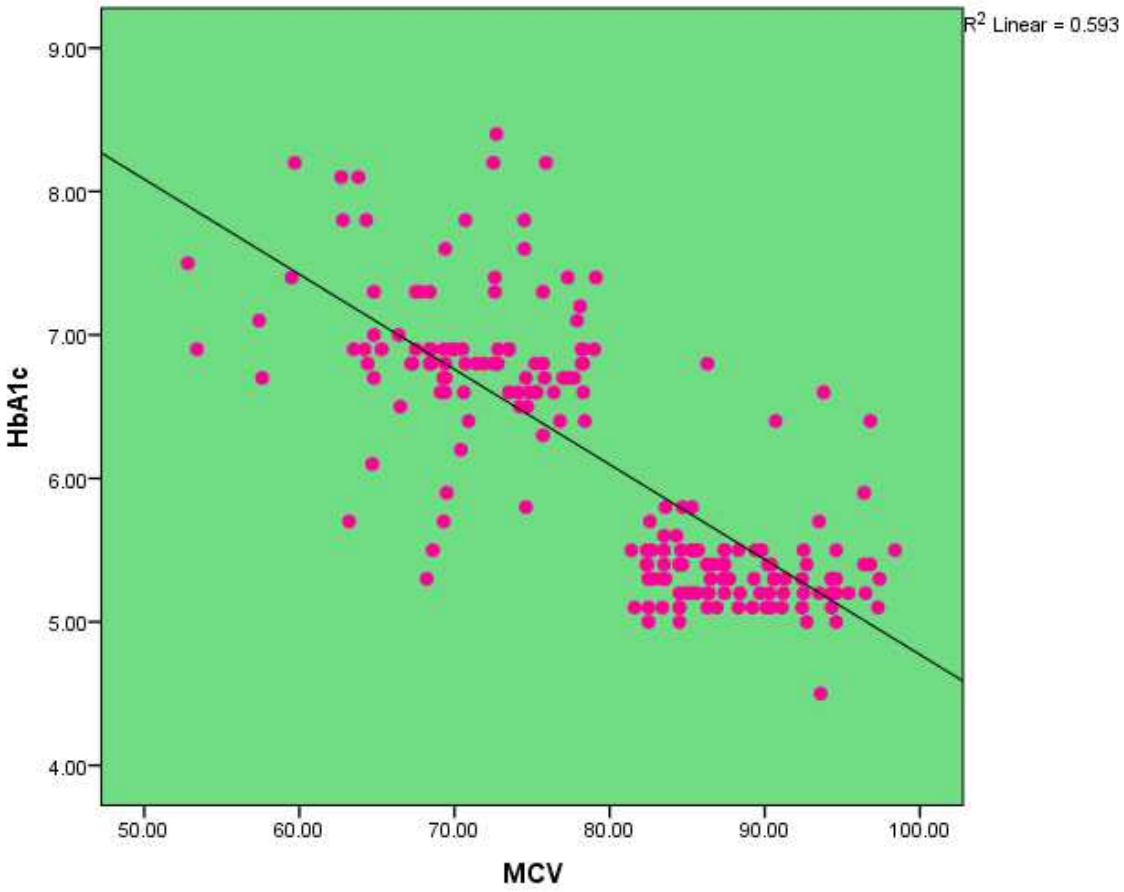
BAR DIAGRAM DEPICTING THE MEAN HbA_1c

The HbA_1c of the cases was 6.89 ± 0.55 % with a calculated t-statistic of 24.573

The HbA_1c of the cases was 5.35 ± 0.28 % with a calculated t-statistic of 24.573

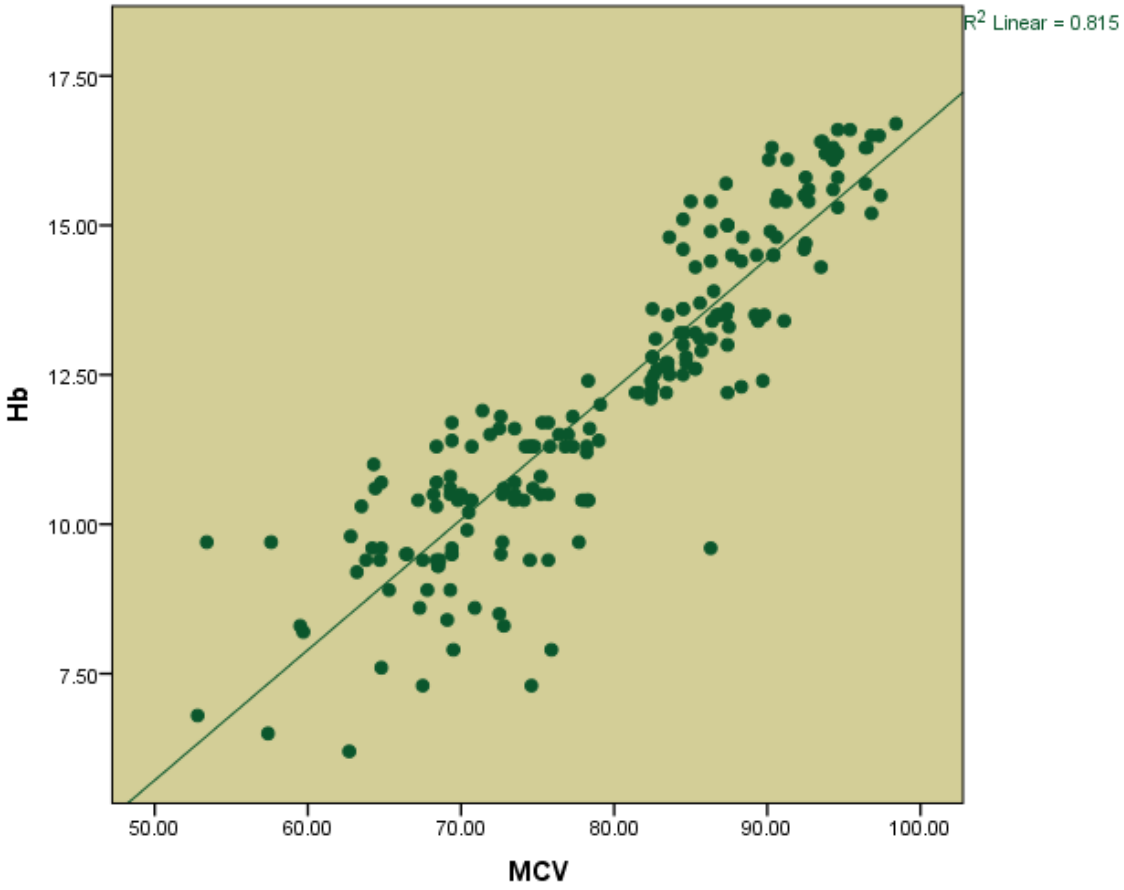


SCATTER PLOT BETWEEN MCV AND HbA_1c



This scatter plot shows the inverse correlation between MCV and HbA_1c.

SCATTER PLOT BETWEEN MCV AND Hb



This scatter plot shows the positive correlation between MCV and Hb.

PEARSON CORRELATION

		MCV	HbA1c	Hb
MCV	**Pearson Correlation**	1	-.770**	.903**
	Sig. (2-tailed)		.000	.000
	N	200	200	200
**. Correlation is significant at the 0.01 level (2-tailed).				

The Pearson correlation between MCV andHbA_1c is "-0.770" which signifies inverse correlation between the two variables, thus completing the aim of the study.

The Pearson correlation between MCV and Hb is "**0.903"** which signifies positive correlation between the two variables.

DISCUSSION

DISCUSSION

A case control study was conducted in Institute of Internal Medicine at Rajiv Gandhi Government General Hospital, Chennai – 600003 for a period of 6 months from April 2016 – September 2016.

100 cases and 100 controls were chosen from the general medical ward based on the inclusion and exclusion criteria as mentioned above. Fasting and post prandial blood sugars and OGTT were performed to all cases and controls in order to ensure that all those chosen for the study were only non – diabetic adults. After getting informed consent from them, all patients were subjected to detailed history taking, physical examination and relevant laboratory investigations. HbA_1c levels was obtained from both the groups and compared using the independent t test.

HbA_1c is commonlyused to assess the long-term blood sugar control in diabetic patients, because $HbA_1c$is a predictor of the risk for the diabetes – related complications, which has been shown by various studies.

Iron deficiency anemia is the most common form of microcytic anemia and is the most prevalentanaemia worldwide. HbA_1c is aglycated haemoglobin used to assess the glycemic status of patients with diabetes over the previous 3 months. Besides blood sugar, variousother conditions such as hemolyticanemias, hemoglobinopathies, hyperbilirubinemias,

iron, folate and vitamin B_{12}deficiency, acute and chronic blood loss, pregnancy, chronic liver disease and uremia affect the levels of HbA_1c. Recently, interest has arisen in studying the variations in HbA_1c levelsthat are encountered in microcytic anemias like iron deficiency anemia.

The earliest study that investigated the effects of iron deficiency anemia on HbA_1c levels was conducted by Brooks et al.,[47]in which HbA_1c levels were assessed in 35 non-diabetic patients with iron deficiency anemia. Assessment of HbA_1c valueswas done before and after treatment with iron. The observation was that HbA_1c levels in iron deficiency anemia patients were higher and the HbA_1c values decreased after treatment with iron.

It was proposed that, iron deficiency alters the quaternary structure of the hemoglobin molecule. Theglycation of the globin chain, in the relative absence of iron would occur more readily[22]. Sluiter et al.,[46] tried to provide an explanation for the above mentionedfindings. They had proposed that the formation of glycated haemoglobin (HbA_1c) within an erythrocyte was an irreversible process. Hence, the concentration of HbA_1c within a singleerythrocyte would linearly increase with the age of the red cell. They also found out that in patients with euglycemia, but with very young red cells, a picture similar to that as would be found after

treatment of iron deficiency anemia, the HbA_1c concentration was reduced.

Later, van Heyningen et al., reported that there was no differences in HbA_1c concentrations compared to the non-diabetic patients with iron deficiency anemia before and after treatmentiron[48]. They also believed that the differences in HbA_1c concentrations that were noted before and after iron supplementation were chiefly due to the differences in the laboratory methods used for measuring and calibrating HbA_1c.

Raiet al., later investigated and came up with the conclusion that even though different methods are usedto measure HbA_1c, no difference wasnoted among the colorimetric methods, ion-exchangechromatography and affinity chromatography[51].

Hansen et al. also demonstrated that HbA_1c levels tend to decreased upon treatment of the anemia[53] adding evidence to the study done by Sluiter et al., This was thought to occur as a result of increased bone marrow erythropoiesis which was brought about by the treatment with iron, thus leading to production of new immature erythrocytes from the bone marrow.They also showed that HbA_1cconcentrations was normal in iron deficiency, which would dropdown tosubnormal levels after iron supplementation.

Further studies conducted by El-Agouza et al., and Cogan et al.,[49] came up with the result stating that HbA_1c levels were higher in patients with iron deficiency anemia and would decrease on iron supplementation[58]. They argued that, elevated HbA_1c levels in iron deficiency anemia was due to the fact that, if the serum glucose remains constant, a decrease in thehemoglobin concentration increasesthe glycation of haemoglobin.

As is evident from the above studies, the mechanisms by which iron deficiency (microcytic)anemia affects HbA_1c levels remains unclear. Since there are a large variations in the study results, we conducted our own study to investigate the effects of microcytic anaemia on HbA_1c levels[60].

In our study, 100 non diabetic cases and 100 non diabetic controls were selected and grouped based mainly on Hb and MCV values. Later HbA_1c values were obtained from both the groups were compared using the independent t test. The correlation between MCV and HbA_1c values was calculated using the Pearson co-efficient. **The 'p' value of the test was <0.001, which is statistically significant with a Pearson co-efficient of "-0.773"**. So our study concludes that an inverse correlation exists between MCV values and HbA_1c levels. So anemiaif present, must be corrected before any decision, whether diagnostic or therapeutic,is made based on the HbA_1c levels.

LIMITATIONS

LIMITATIONS

Our observations, since being made in a government general hospital, the study population chiefly belonged to a lower socio-economic class. Here, the causes of microcytic anemia, the major cause being iron deficiency anemia is not just bleeding and malabsorption, but also nutritional deficiency which may also have an impact on our study results. Other unknown variables such as racial, geographical factors also may influence our results. Further studies to confirm the roles of various other factors are needed to confirm our findings.

CONCLUSION

CONCLUSION

Microcytic anemia definitely has an impact on the HbA_1c. As MCV decreases in patients with microcytic anemia, HbA_1c tends to rise.

Thus, microcytic anemia, if present must be corrected before making any diagnostic or therapeutic decision based on the HbA_1c levels.

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ANNEXURES

PROFORMA

Name: Age/Sex:
Address: Occupation:

SYMPTOMS:

Easy fatiguability	
Abdominal pain	
Abdominal distension	
Dyspnea	
Pedal edema	
Previous blood transfusions	
Malena	

PAST HISTORY:

CAD	
CKD	
CLD	

PERSONAL HISTORY:

SMOKING
ALCOHOL

FAMILY HISTORY OF DIABETES:

GENERAL EXAMINATION:

Pallor
Icterus
Cyanosis
Clubbing
Pedal edema
Lymphadenopathy

BMI:

VITAL SIGNS:

PR-
BP-
RR-
JVP-
Temp-

SYSTEMIC EXAMINATION:

CVS:

RS:

ABDOMEN:

CNS:

INVESTIGATIONS:

COMPLETE HEMOGRAM:

Hemoglobin:
MCV:
MCH:
MCHC:
Hematocrit:
ESR:

PERIPHERAL SMEAR:

FASTING BLOOD GLUCOSE (FBG):

POST PRANDIAL BLOOD GLUCOSE (PPBG):

ORAL GLUCOSE TOLERANCE TEST (OGTT): Fasting:
1 hr:
2hr:

HbA1c:

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr.Gokhula Raj.B.
Post Graduate in M.D. General Medicine
Madras Medical College
Chennai 600 003

Dear Dr.Gokhula Raj.B.,

The Institutional Ethics Committee has considered your request and approved your study titled **"EFFECT OF MICROCYTIC ANEMIA ON GLYCOSYLATED HEMOGLOBIN A1c IN NON-DIABETIC ADULTS " - NO.06032016.**

The following members of Ethics Committee were present in the meeting hold on **01.03.2016** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
2.Dr.R.Vimala,MD.,Dean,MMC,Ch-3	:Deputy Chairperson
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9.Tmt.J.Rajalakshmi, JAO,MMC, Ch-3	: Lay Person
10.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
11.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary - Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

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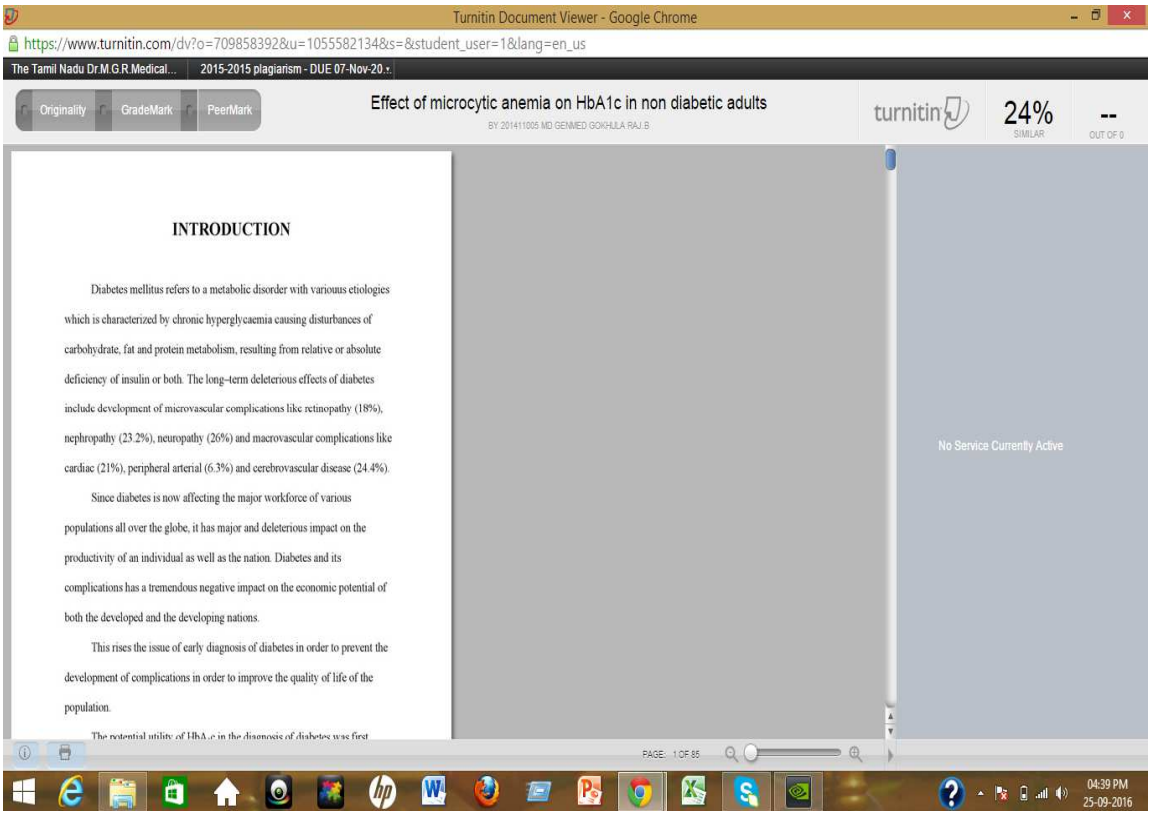

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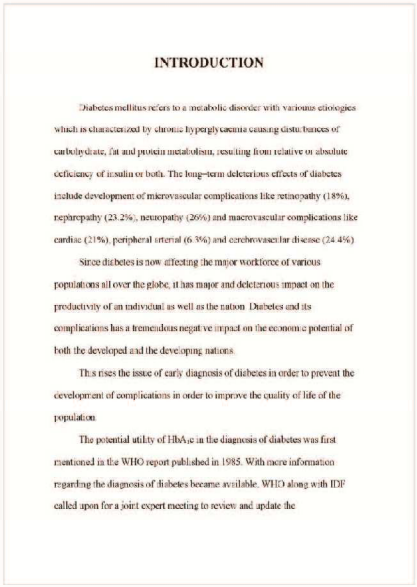

INTRODUCTION

Diabetes mellitus refers to a metabolic disorder with various etiologies which is characterized by chronic hyperglycaemia causing disturbances of carbohydrate, fat and protein metabolism, resulting from relative or absolute deficiency of insulin or both. The long-term deleterious effects of diabetes include development of microvascular complications like retinopathy (18%), nephropathy (23.2%), neuropathy (26%) and macrovascular complications like cardiac (21%), peripheral arterial (6.3%) and cerebrovascular disease (24.4%)

Since diabetes is now affecting the major workforce of various populations all over the globe, it has major and deleterious impact on the productivity of an individual as well as the nation. Diabetes and its complications has a tremendous negative impact on the economic potential of both the developed and the developing nations.

This rises the issue of early diagnosis of diabetes in order to prevent the development of complications in order to improve the quality of life of the population.

The potential utility of HbA_{1c} in the diagnosis of diabetes was first mentioned in the WHO report published in 1985. With more information regarding the diagnosis of diabetes became available, WHO along with IDF called upon for a joint expert meeting to review and update the

INFORMATION SHEET

We are conducting a study on **"EFFECT OF MICROCYTIC ANEMIA ON GLYCOSYLATED HEMOGLOBIN A_1c IN NON-DIABETIC ADULTS"** among patients attending Rajiv Gandhi Government General Hospital, Chennai and for that your specimen may be valuable to us.

The purpose of this study is to estimate HbA_1c levels in non-diabetic adults with iron deficiency anemia and to compare it with HbA_1c levels of non-diabetic controls.

We are selecting certain cases and if you are found eligible, we may be using your blood samples to do certain tests which in any way do not affect your final report or management.

The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of Investigator	Signature of Participant
Date: Place:	

ஆராய்ச்சி தகவல் தாள்

சென்னை ராஜீவ்காந்தி அரசு பொது மருத்துவமனையின் பொது மருத்துவத்துறையில் "நீரிழிவு நோய் அல்லாதவர்களில் குளுகோஸ் கூட்டப்பட்ட ஹீமோகுளோபின் ஏ1சியின மீது குறைந்த சிகப்பு ரத்த அணுக்களின் கொள்ளளவின் விளைவை ஆராய்தல்" பற்றிய ஆய்வு நடைபெறுகிறது.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இதனால் தங்களது சிகிச்சையில் பாதிப்பு ஏற்படாது என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்த ஆய்வில் தங்களுக்கு மருத்துவபரிசோதனை, இரத்த வட்டுக்களின் கொள்ளவு ஆல்புமினூரியா, ஹீமோகுளோபின் ஏ1சி உள்ளிட்ட இரத்தப் பரிசோதனை மற்றும் சிறுநீர் பரிசோதனை செய்யப்படும்.

முடிவுகளை அல்லது கருத்துக்களை வெளியிடும்போதோ அல்லது ஆராய்ச்சியின்போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிட மாட்டோம் என்பதை தெரிவித்துக்கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின்பேரில்தான் இருக்கிறது. மேலும் நீங்கள் எந்த நேரமும் இந்த ஆராய்ச்சியிலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்த சிறப்பு பரிசோதனைகளின் முடிவுகளையும் நோயின் தன்மை பற்றியும் ஆராய்ச்சியின்போது அல்லது ஆராய்ச்சியின் முடிவின்போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

ஆராய்ச்சியாளர் கையொப்பம் **பங்கேற்பாளர் கையொப்பம்**

நாள் :
இடம் :

CONSENT FORM

Study Detail : **"EFFECT OF MICROCYTIC ANEMIA ON GLYCOSYLATED HEMOGLOBIN A_{1c} IN NON-DIABETIC ADULTS"**

Study Centre : RajivGandhiGovernment GeneralHospital, Chennai.

Patient's Name :

Patient's Age :

Identification Number :

Patient may check (√) these boxes

a) I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask question and all my questions and doubts have been answered to my complete satisfaction. ❑

b) I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected. ❑

c) I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study. ❑

d) I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperate with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or well being or any unexpected or unusual symptoms. ❑

e) I hereby consent to participate in this study. ❑

f) I hereby give permission to undergo detailed clinical examination and blood investigations as required. ❑

Signature/thumb impression

Signature of Investigator

Patient's Name and Address:

Study Investigator's Name:
Dr. GOKHULA RAJ.B

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சியின் தலைப்பு

நீரிழிவு நோய் அல்லாதவர்களில் குளுகோஸ் கூட்டப்பட்ட ஹீமோகுளோபின் ஏ1சியின மீது குறைந்த சிகப்பு ரத்த அணுக்களின் கொள்ளளவின் விளைவை ஆராய்தல்

ஆய்வு நிலையம் : பொது மருத்துவத்துறை,
சென்னை மருத்துவக் கல்லூரி சென்னை – 3.

பங்கு பெறுபவரின் பெயர் :

உள்நோயாளி எண் :

பங்குபெறுபவர் இதனை (✓) குறிக்கவும்

மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டது. என்னுடைய சந்தேகங்களை கேட்கவும், அதற்கான தகுந்த விளக்கங்களை பெறவும் வாய்ப்பளிக்கப்பட்டது. ☐

நான் இவ்வாய்வில் தன்னிச்சையாகதான் பங்கேற்கிறேன். எந்த காரணத்தினாலோ எந்த கட்டத்திலும் எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகி கொள்ளலாம் என்றும் அறிந்து கொண்டேன். ☐

இந்த ஆய்வு சம்பந்தமாகவோ, இதை சார்ந்த மேலும் ஆய்வு மேற்கொள்ளும் போதும் இந்த ஆய்வில் பங்குபெறும் மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளை பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். நான் ஆய்வில் இருந்து விலகிக் கொண்டாலும் இது பொருந்தும் என அறிகிறேன். ☐

இந்த ஆய்வின் மூலம் கிடைக்கும் தகவல்களையும், பரிசோதனை முடிவுகளையும் மற்றும் சிகிச்சை தொடர்பான தகவல்களையும் மருத்துவர் மேற்கொள்ளும் ஆய்வில் பயன்படுத்திக்கொள்ளவும் அதை பிரசுரிக்கவும் என் முழு மனதுடன் சம்மதிக்கின்றேன். ☐

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக்கொள்கிறேன். எனக்கு கொடுக்கப்பட்ட அறிவுரைகளின்படி நடந்து கொள்வதுடன், இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்று உறுதியளிகிறேன். ☐

இந்த இரு அறுவை சிகிச்சை முறைகளும் ஒப்புக்கொள்ளப்பட்ட முறைகள் என்பதையும் இதனால் உடலுக்கு எந்தவிதமான உபாதைகளும் இருக்காது என்பதை அறிந்துகொண்டு இந்த ஆய்வில் பங்குபெற முழு மனதுடன் சம்மதிக்கிறேன். ☐

பங்கேற்பவரின் கையொப்பம் ..... இடம்..... தேதி.....

இடது கை பெருவிரல் ரேகை

பங்கேற்பவரின் பெயர் மற்றும் விலாசம் .....

ஆய்வாளரின் கையொப்பம் ..... இடம்..... தேதி.....

ஆய்வாளரின் பெயர் .....

MASTER CHART

SERIAL NO.	CATEGORY	AGE	SEX	FBS	PPBS	OGTT (2 hrs)	Hb	MCV	HbA1c
1	CASE	45	M	97	116	120	10.5	72.7	6.8
2	CASE	34	M	76	107	104	9.5	66.5	6.5
3	CASE	57	F	89	121	115	9.4	68.6	5.5
4	CASE	47	M	98	135	124	6.5	57.4	7.1
5	CASE	38	M	92	120	116	8.5	72.5	8.2
6	CASE	78	F	89	136	119	11.5	76.4	6.6
7	CASE	34	M	78	123	129	12.4	78.3	6.6
8	CASE	65	M	87	104	114	11.2	78.2	6.8
9	CASE	34	M	84	109	104	8.3	72.8	6.9
10	CASE	35	F	94	137	107	9.2	63.2	5.7
11	CASE	65	M	78	113	114	9.7	57.6	6.7
12	CASE	43	M	84	106	104	10.6	74.7	6.5
13	CASE	42	F	80	122	118	11.4	79	6.9
14	CASE	76	F	94	121	127	9.6	64.2	6.9
15	CASE	73	F	75	109	108	8.9	67.8	7.3
16	CASE	56	F	85	116	114	7.9	69.5	5.9
17	CASE	52	F	93	118	131	8.6	70.9	6.4
18	CASE	56	M	67	104	103	10.4	78.3	6.8
19	CASE	62	M	75	109	120	9.7	77.7	6.7
20	CASE	38	F	72	115	118	9.3	68.5	6.9
21	CASE	42	M	79	132	112	10.8	75.2	6.6
22	CASE	38	F	84	106	107	11.3	76.8	6.4
23	CASE	63	F	78	118	136	9.7	72.7	8.4
24	CASE	53	M	83	105	103	12	79.1	7.4
25	CASE	28	M	92	114	106	11.7	75.3	6.6
26	CASE	48	F	86	108	114	10.6	72.8	6.8
27	CASE	53	F	85	128	119	9.8	62.8	7.8
28	CASE	72	M	72	119	124	11.4	69.4	6.7
29	CASE	58	F	75	124	105	10.4	73.5	6.9
30	CASE	68	M	81	114	109	9.7	53.4	6.9
31	CASE	46	F	90	107	113	7.9	75.9	8.2
32	CASE	55	F	74	114	101	10.5	69.3	6.7
33	CASE	60	M	84	117	108	11.3	68.4	6.8
34	CASE	34	F	93	109	124	9.4	74.5	7.6
35	CASE	72	M	69	114	127	10.6	64.4	6.8
36	CASE	50	M	78	123	118	11.7	69.4	6.6
37	CASE	37	F	70	118	108	10.4	77.9	7.1
38	CASE	52	M	89	129	103	6.8	52.8	7.5
39	CASE	47	F	79	108	134	10.4	78.3	6.9
40	CASE	58	F	82	103	115	7.6	64.8	7
41	CASE	40	F	90	123	107	9.5	72.6	7.4
42	CASE	36	F	75	133	108	11.3	75.8	6.7
43	CASE	52	M	84	114	113	7.3	67.5	6.9
44	CASE	45	F	76	124	108	11.8	72.6	7.3
45	CASE	69	M	83	113	117	8.2	59.7	8.2
46	CASE	70	M	96	135	109	10.6	69.3	6.7
47	CASE	38	M	74	113	131	11.9	71.4	6.8
48	CASE	46	F	80	125	119	10.3	68.4	6.9
49	CASE	26	F	64	120	113	9.6	64.8	7.3
50	CASE	36	M	79	119	124	9.4	75.7	6.3

51	CASE	35	M	78	128	104	7.3	74.6	6.7
52	CASE	56	F	87	116	115	8.3	59.5	7.4
53	CASE	57	F	83	105	135	10.5	75.2	6.8
54	CASE	74	M	67	138	114	11	64.3	7.8
55	CASE	35	F	79	123	124	11.5	77	6.7
56	CASE	57	M	63	118	105	10.4	67.2	6.8
57	CASE	65	F	79	108	103	9.4	67.5	7.3
58	CASE	27	M	85	129	118	8.9	69.3	5.7
59	CASE	74	F	63	114	104	11.6	78.4	6.4
60	CASE	45	F	84	105	118	10.7	73.5	6.9
61	CASE	75	M	73	130	105	11.3	74.5	7.8
62	CASE	56	M	69	114	114	9.6	69.4	7.6
63	CASE	24	F	66	112	117	10.5	70	6.9
64	CASE	47	M	72	132	126	11.3	77.3	6.7
65	CASE	48	F	84	104	106	9.4	64.7	6.1
66	CASE	38	M	59	121	108	11.3	74.2	6.5
67	CASE	48	F	93	108	118	10.8	69.3	6.9
68	CASE	56	M	73	115	125	9.4	68.4	7.3
69	CASE	27	F	95	122	130	10.3	63.5	6.9
70	CASE	36	F	78	104	113	10.5	68.2	5.3
71	CASE	46	F	72	119	108	11.3	70.7	6.8
72	CASE	54	M	69	120	115	9.6	86.3	6.8
73	CASE	54	F	83	129	132	10.2	70.5	6.9
74	CASE	48	M	82	105	104	11.3	78.2	6.9
75	CASE	37	F	63	118	122	10.5	75.7	7.3
76	CASE	73	M	84	115	118	6.2	62.7	8.1
77	CASE	54	F	78	136	105	11.6	72.5	6.8
78	CASE	56	M	75	104	114	8.9	65.3	6.9
79	CASE	67	M	89	126	104	9.5	66.4	7
80	CASE	32	F	83	117	118	10.7	64.8	6.7
81	CASE	56	M	93	104	103	11.5	71.9	6.8
82	CASE	37	M	92	137	124	9.9	70.4	6.2
83	CASE	73	F	96	113	117	11.3	74.6	5.8
84	CASE	38	M	82	104	132	10.4	74.1	6.6
85	CASE	63	F	73	107	116	9.5	69.4	6.8
86	CASE	28	F	58	119	104	10.7	68.4	6.9
87	CASE	48	F	74	121	124	11.3	74.8	6.6
88	CASE	38	M	83	116	119	11.8	77.3	7.4
89	CASE	57	M	60	120	104	10.4	78.1	7.2
90	CASE	59	F	85	114	126	8.6	67.3	6.8
91	CASE	61	F	72	102	114	10.4	69.8	6.9
92	CASE	47	M	83	116	126	10.4	70.6	6.6
93	CASE	47	F	95	108	117	9.4	63.8	8.1
94	CASE	34	M	73	104	104	11.7	75.7	6.8
95	CASE	43	M	76	118	112	10.5	73.5	6.6
96	CASE	27	M	89	125	107	11.6	73.5	6.9
97	CASE	59	F	85	105	115	9.5	69.4	6.7
98	CASE	24	F	91	105	106	10.4	70.7	7.8
99	CASE	59	F	76	113	115	9.3	68.5	6.8
100	CASE	77	M	85	110	104	8.4	69.1	6.6

SERIAL NO.	CATEGORY	AGE	SEX	FBS	PPBS	OGTT (2hrs)	Hb	MCV	HbA1c
1	CONTROL	20	F	75	110	103	12.2	87.4	5.5
2	CONTROL	25	M	72	126	118	16.3	96.5	5.2
3	CONTROL	64	M	85	120	125	14.5	90.4	5.4
4	CONTROL	36	F	78	100	135	13.5	89.2	5.1
5	CONTROL	55	M	84	108	116	15.5	90.7	6.4
6	CONTROL	70	F	75	134	100	13.2	84.3	5.6
7	CONTROL	63	M	78	125	131	16.1	91.3	5.3
8	CONTROL	60	F	83	130	107	12.4	89.7	5.2
9	CONTROL	43	F	92	105	115	14.3	85.3	5.5
10	CONTROL	28	F	91	123	137	13.5	86.9	5.1
11	CONTROL	50	F	70	137	128	13.1	85.6	5.5
12	CONTROL	53	M	85	112	118	14.8	83.6	5.8
13	CONTROL	69	M	88	115	125	15.4	91.2	5.2
14	CONTROL	60	F	76	135	101	12.7	83.5	5.5
15	CONTROL	45	M	90	120	117	16.1	94.3	5.3
16	CONTROL	35	M	95	127	135	16.2	93.8	6.6
17	CONTROL	26	M	77	100	128	14.5	90.4	5.1
18	CONTROL	58	F	81	105	125	13.6	84.5	5.1
19	CONTROL	60	M	89	110	110	15.7	96.4	5.9
20	CONTROL	45	F	98	135	138	12.8	82.5	5.5
21	CONTROL	30	M	95	122	132	14.5	87.7	5.3
22	CONTROL	37	M	70	135	126	16.1	90.1	5.1
23	CONTROL	62	F	75	131	138	13.7	85.6	5.2
24	CONTROL	70	M	95	106	133	15	87.4	5.2
25	CONTROL	22	F	72	118	112	13.5	83.5	5.6
26	CONTROL	38	M	86	107	115	14.9	86.3	5.4
27	CONTROL	45	F	84	113	102	12.2	81.4	5.5
28	CONTROL	56	F	90	121	125	13.6	84.5	5
29	CONTROL	76	F	74	136	106	13.1	82.7	5.5
30	CONTROL	50	M	87	100	136	16.3	96.4	5.4
31	CONTROL	54	M	88	125	139	15.8	92.5	5.2
32	CONTROL	56	M	90	109	128	15.4	90.6	5.3
33	CONTROL	62	M	95	105	123	14.3	93.5	5.2
34	CONTROL	48	F	85	98	104	12.6	85.3	5.5
35	CONTROL	50	M	70	138	118	15.6	92.7	5.4
36	CONTROL	68	F	76	104	105	12.4	82.4	5.5
37	CONTROL	33	F	84	125	125	12.7	84.7	5.8
38	CONTROL	75	M	89	133	120	16.5	97.3	5.1
39	CONTROL	55	F	92	127	114	14.6	84.5	5.4
40	CONTROL	42	M	75	130	139	16.2	94.6	5.5
41	CONTROL	65	M	73	119	126	15.4	92.7	5
42	CONTROL	36	M	72	129	135	16.5	96.8	6.4
43	CONTROL	25	F	75	126	110	13.4	86.4	5.2
44	CONTROL	78	M	88	130	100	15.6	94.3	5.1
45	CONTROL	70	F	95	106	113	13.5	86.7	5.4
46	CONTROL	23	F	77	100	124	12.6	82.8	5.3
47	CONTROL	78	F	82	136	136	12.8	82.5	5
48	CONTROL	57	M	87	132	125	15.3	94.6	5.3
49	CONTROL	64	M	96	101	131	14.7	92.5	5.5
50	CONTROL	36	F	76	113	119	12.5	83.6	5.3

51	CONTROL	59	M	71	104	138	16.3	90.3	5.2
52	CONTROL	60	F	83	117	108	13.4	89.4	5.5
53	CONTROL	44	M	94	115	120	14.9	90.2	5.4
54	CONTROL	50	M	95	109	102	15.8	94.6	5
55	CONTROL	28	F	80	127	137	12.6	85.3	5.8
56	CONTROL	43	F	86	134	123	12.2	81.6	5.1
57	CONTROL	46	F	75	138	139	13	87.4	5.5
58	CONTROL	66	M	72	120	113	14.5	89.3	5.3
59	CONTROL	54	M	86	124	105	15.7	87.3	5.4
60	CONTROL	77	F	80	135	100	13.6	82.5	5.1
61	CONTROL	51	M	78	119	122	15.1	84.5	5.1
62	CONTROL	64	F	76	114	131	13	84.5	5.4
63	CONTROL	75	M	85	120	112	16.6	94.6	5.2
64	CONTROL	80	F	83	128	104	12.5	82.6	5.7
65	CONTROL	45	F	98	130	118	12.2	82.4	5.4
66	CONTROL	62	M	94	121	111	14.4	88.3	5.5
67	CONTROL	49	F	73	111	101	13.3	87.5	5.3
68	CONTROL	33	M	70	123	128	16.1	94.3	5.2
69	CONTROL	60	F	84	131	132	13.1	86.3	5.4
70	CONTROL	45	F	75	138	115	12.3	88.3	5.1
71	CONTROL	67	F	73	105	109	12.9	85.7	5.5
72	CONTROL	77	M	79	125	110	15.5	97.4	5.3
73	CONTROL	70	M	86	116	122	15.2	96.8	5.4
74	CONTROL	40	F	95	122	133	13.4	91.1	5.1
75	CONTROL	25	M	91	130	107	16.7	98.4	5.5
76	CONTROL	78	M	81	135	121	14.8	88.4	5.2
77	CONTROL	54	F	76	101	126	13.2	84.6	5.5
78	CONTROL	50	M	79	109	120	14.4	86.3	5.2
79	CONTROL	38	F	87	128	135	12.6	83.5	5.4
80	CONTROL	67	M	84	125	114	15.4	86.3	5.1
81	CONTROL	66	M	73	120	106	16.3	94.3	5.3
82	CONTROL	48	F	96	100	102	12.2	83.4	5.1
83	CONTROL	58	F	77	115	132	13.5	87.3	5.3
84	CONTROL	60	F	70	124	112	12.5	84.5	5.2
85	CONTROL	65	M	73	139	108	15	87.4	5.4
86	CONTROL	33	M	80	122	103	14.6	92.4	5.3
87	CONTROL	73	F	85	132	126	13.6	87.4	5.4
88	CONTROL	35	M	88	114	128	16.6	95.4	5.2
89	CONTROL	42	F	74	133	130	12.1	82.4	5.4
90	CONTROL	44	M	79	120	115	16.4	93.6	4.5
91	CONTROL	80	F	87	125	134	12.7	83.4	5.3
92	CONTROL	75	F	85	130	126	13.2	85.3	5.2
93	CONTROL	31	M	72	118	105	16.4	93.5	5.7
94	CONTROL	62	F	75	105	124	13.9	86.5	5.3
95	CONTROL	55	M	85	110	122	15.4	85	5.2
96	CONTROL	70	F	84	137	134	12.3	82.5	5.3
97	CONTROL	32	F	96	128	118	12.8	84.7	5.4
98	CONTROL	48	F	73	113	115	13.5	89.8	5.5
99	CONTROL	62	M	81	132	135	14.8	90.6	5.3
100	CONTROL	60	M	95	128	120	15.5	92.4	5.1